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Nicolas G. Bazan, M.D., Ph.D.
Project Director

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Neuropharmacology of Delta Receptor Agonists and Antagonists

Project Directors:
Joseph Moerschbaecher, Ph.D.

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ABSTRACT

Studies in the Division of Neuropharmacology investigated the role of endogenous opioid systems in learning and memory, ventilatory function and antinociception. The goal of these studies was: to identify and characterize candidate ligands that might be useful in studies on *delta* opioid mechanisms; and to use these compounds to systematically investigate the role of *delta* systems in complex behavioral processes, in respiration and in the perception of noxious stimuli. The first candidate compound was BW373U86, which is a highly-selective agonist for the *delta* opioid receptor. BW373U86 had effects that differed from those of other prototypic opioid agonists. BW373U86 failed to produce antinociceptive effects in rhesus monkeys. Although BW373U86 had effects that were unique from the effects obtained with prototypic *mu* or *kappa* opioid agonists, the behavioral and pharmacological profile for this agonist was disappointing. A second candidate compound which was focused upon was OHM3507. This compound differs from morphine in that it does not suppress the immune system. It was hypothesized that this differences might be due to activity at the delta opioid receptor. The pharmacologic and behavioral effects of the fentanyl derivative OHM3507 were assessed to determine if this compound had increased antinociceptive effects and a reduced number of undesirable effects (e.g., respiratory depression) as compared to the prototypic opioids (e.g., fentanyl, morphine). Studies were undertaken to determine the opioid receptor selectivity of OHM3507 using behavioral assays in rhesus monkey. At a dose of 0.32 mg/kg, OHM3507 produced 100% antinociception (20s latency) in monkeys and reduced minute volume respiration to <60% in both air and 5% CO_2 in O_2. In subjects treated daily with morphine (3.2 mg/kg) discriminating between saline and 0.01 mg/kg naltrexone, OHM3507 attenuated responding on the naltrexone-associated lever in a dose-dependent manner. OHM3507 decreased

the rate of responding at doses greater than 0.1 mg/kg, but did not disrupt learning or performance of a complex behavioral task in monkeys. The administration of naltrexone dose-dependently shifted the OHM3507 dose-effect curve to the right with pA_2s of 7.8 and 8.2 for antinociception and discrimination, respectively. OHM3507 produced 100% antinociception at 3.2 mg/kg. These data suggest that: 1) OHM3507 produces fentanyl-like effects at the μ opioid receptor in rhesus monkeys, in contrast to its effects in rodents; 2) the reported lack of NK-cell activity after OHM3507 administration suggests a reduced risk of immunosuppression produced by this chemical class and 3) there is a necessity to study this class of compounds under a variety of experimental conditions in different species in order to better characterize the properties of these compounds and their potential clinical values, however, our studies indicate that they are devoid of *delta* opioid receptor activity. The highly selective nonpeptidic ligand, SNC-80 was the third candidate compound investigated. This compound is the methyl ether of one enantiomer of BW373U86, but differs from BW373U86 in that it is systemically active and has demonstrated selectivity for the *delta* receptor that is comparable to that seen with *delta*-selective opioid peptides. In the ventilation studies, 0.1-1 mg/kg of SNC-80 decreased all three measures of respiration in air in a dose-related manner. Interestingly, and in contrast to the effects of some typical *mu* opioids, the effects of SNC-80 on ventilation in air were larger than the effects on ventilation in 5% CO_2. At a dose of 1 mg/kg, SNC-80 decreased minute volume in air to less than 50% of control in air. In antinociceptive studies, doses of SNC-80 as high as 3.2 mg/kg failed to produce an antinociceptive effect in any of the four rhesus monkeys studied. This lack of antinociceptive effect with SNC-80 were consistent with results obtained previously with BW373U86. Studies also determined whether a similar range of doses of SNC-80 could disrupt measures of rate and accuracy in monkeys responding under a

multiple schedule of learning and performance. In each of the monkeys tested , SNC-80 (0.1-5.6 mg/kg) dose-dependently decreased the overall rate of responding in both components, and selectively decreased the accuracy of responding in the learning component. The effects of SNC-80 on the accuracy of responding were selective in that it produced small error-increasing effects in acquisition, but failed to produce any error-increasing effects in performance across the same dose range. In summary, these data suggest that SNC-80 produces BW373U86-like behavioral effects at *delta* opioid receptors in rhesus monkeys. The potential clinical or therapeutic value of *delta* agonists may reside with their interaction with *mu* opioid receptor systems, because *delta* opioid agonists are similar to *mu* opioids on measures of respiration and complex behavioral processes but devoid of analgesic effects in rhesus monkeys.

INTRODUCTION

Delta opioid receptors remain one of the most interesting and unexplored classes of opioid receptor. In addition, *delta* opioids are thought to have considerable therapeutic potential, although to date there are no approved indications for *delta* agonists or antagonists. The clinical advantages that are presumed to be associated with *delta* opioid agonists, as compared to compounds that act at other types of opioid receptors, are: reduced abuse liability; reduced dependence potential; and reduced toxicity (Cheng et al., 1993; Cowan et al., 1988; Porreca et al., 1983). The data indicating this improved clinical profile has largely come from studies with rodents, where it has been shown that *delta* receptor agonists have robust antinociceptive effects in the absence of significant respiratory depression. This pharmcologic profile, which is in direct contrast to the profile of many prototypical mu opioids, is particularly promising for the development of new analgesics.

The 4-heteroanilido-piperidine class is a class of compounds which seems to be characterized by compounds having novel combinations of opioid and non-opioid receptor mediated effects. One of these derivatives, mirfentanil, has been studied extensively and has low efficacy at μ opioid receptors compared to fentanyl and morphine in rodents. Mirfentanil produces antinociceptive effects and a limited amount of respiratory depressive effects, but reverses morphine-induced antinociception and respiratory depression (Wynn et al, 1994). In contrast, mirfentanil, in rhesus monkeys, produces some antinociceptive effects that do not appear to be mediated by μ, κ, or δ opioid receptors (France, et al., 1991), since its antinociceptive effects are not antagonized by naltrexone, and produces little to no respiratory depressant effects (France et al., 1991) suggesting a possible non-opioid mechanisms for this compound.

Another member of this class, OHM3507, is a low efficacy opioid agonist in rodents and rabbits; OHM3507 produces modest antinociceptive effects in the rabbit tooth pulp assay and reverses morphine-induced respiratory depression and antinociception in rabbits (Wynn *et al.*, 1991). This profile of effects is similar to mirfentanil and suggests that OHM3507 may be a novel compound of the same type as mirfentanil, producing antinociception with a decreased amount of respiratory depressant effects, which would increase the therapeutic potential of the compound. However, one characteristic becoming apparent in this class of compounds is the differentiation of effects seen with these compounds in different species. OHM3568, for example, has low efficacy in producing antinociception in rodents, and has a pharmacological profile nearly identical to fentanyl in non-human primates (France *et al.*, 1992), while OHM3295 is a low efficacy opioid agonist in non-human primates and in rodents (France *et al.*, 1995). Because of the varying responses obtained among different species with this family of compounds, systemic comparisons among a variety of species appears warranted for these fentanyl derivatives.

During this project period the antinociceptive effects of OHM3507 as well is its respiratory depressant effects and amnesic/dissociative effects were determined in rhesus monkeys. The pharmacologic effects of OHM3507 were characterized using a warm-water tail withdrawal procedure to measure antinociception, pressure changes in a head plethysmograph to measure respiration, and the learning and performance of a discrimination under a food schedule to study complex behavior. Unfortunately, in monkeys, *delta* receptor ligands such as BW373U86 have exhibited relatively poor systemic activity and significant behavioral toxicity including convulsions and "barrel-rolling" behavior (see 1995 Progress Report). Despite these initial findings for certain *delta* receptor ligands, the pharmacologic profile for these opioids on many physiological and

behavioral measures remains unprecedented and extraordinarily important to our understanding of opioid mechanisms and their interactions. For these reasons, the focus of many of the studies conducted previously centered on specific low efficacy *mu* agonists (e.g., OHM3507) and the interactions of opioids with other receptor systems such as the GABA receptor system that may be involved in the control of pain.

Studies on the fentanyl derivative OHM3507 have been completed and are in press (Ahn et al., submitted). Initially, it was hypothesized that the pharmacologic actions of OHM3507 would not be morphine-like in rhesus monkeys and might, in part, involve agonist actions at *delta* opioid receptors. This hypothesis was based on the fact that OHM3507 exhibited a novel spectrum of effects in rodents (Bagley et al., 1989), and was based on effects obtained in non-human primates (France et al., 1991, 1995c) with other compounds in this series that also had novel effects in rodents (e.g., mirfentanil). However, this hypothesis was not supported insofar as OHM3507 had robust agonist actions in a variety of assays and these agonist actions appeared to be exclusively the result of actions at *mu* opioid receptors. These studies have provided another example of the sometimes poor concordance between results obtained in rodents and results obtained in other species. Like mirfentanil, OHM3295 also had novel effects in rodents (Bagley et al., 1989); however, in rhesus monkeys, this fentanyl derivative was essentially identical to the parent compound fentanyl and displayed no unusual pharmacologic actions (France et al., 1992). Several other compounds in this series have been investigated (Baker et al., 1995; France et al., 1995a) using other procedures and

ongoing studies are investigating yet another fentanyl derivative, OHM10579, which is a deuterium-substituted mirfentanil.

In learning and memory studies, OHM3507 displayed pharmacological actions very similar to other *mu* agonists. As reported earlier, OHM3507 disrupted complex behavior in a manner similar to that seen after the administration of morphine, methadone or heroin (Moerschbaecher and Thompson, 1983; Moerschbaecher et al., 1983). That is, in both acquisition and performance components, OHM3507 dose-dependently decreased overall rates of responding while increasing acquisition errors only at doses that substantially decreased response rate in each subject. Of note with OHM3507, however, was the observation that some of the disruptive effects on complex behavioral processes persisted for 24 hr. or longer post-injection.

Studies on opioid and benzodiazepine combinations. During the third budget year a series of studies was conducted which investigated interactions between opioids and benzodiazepines (France et al., 1995b). Individually, benzodiazepines and opioids are used extensively and are prescribed widely for the treatment of anxiety and pain, respectively; however, under some conditions these classes of compounds are co-administered. For example, combinations of opioids and benzodiazepines are used routinely for surgical anesthesia and, in patients with chronic pain (receiving opioids), benzodiazepines are commonly used to treat insomnia, anxiety, muscle tension and pain. The purpose of this study was to evaluate interactive effects between opioids and drugs that act at benzodiazepine receptors since it is generally believed that the therapeutic potential of benzodiazepine agonists, antagonists and inverse agonists has not been fully recognized. A significant number of new therapeutics, which have as their primary mechanism(s) of action either agonism, antagonism or inverse (reverse) agonism at specific types of benzodiazepine receptors,

should become available in the near future. Given the likelihood that opioids and various drugs that act at benzodiazepine receptors will be administered concurrently in some patients, it is critical that potential interactions between these classes of compounds be thoroughly investigated. Moreover, studies with benzodiazepines in combination with *mu* agonists will be used to compare results obtained when benzodiazepines are studied in combination with *delta*-selective ligands.

Several different opioid agonists and benzodiazepines agonists have been studied alone and in combination for their effects on ventilation in rhesus monkeys. When administered alone, both opioid (e.g., alfentanil) and benzodiazepine (e.g., lorazepam) agonists decreased ventilation (frequency [*f*], tidal volume [V_T] and minute volume [V_E]) in monkeys (n=4) breathing air or 5% CO_2; however, the effects of morphine-like opioids increased monotonically up to doses that produced apnea whereas the effects of benzodiazepines reached an asymptote at V_E values between 60 and 90% (in air) of control. Acute pretreatment with lorazepam shifted the alfentanil dose-effect curves for *f* and V_E leftward, although these interactions were not greater than additive. Among the set of compounds that have been studied thus far, the interactions between *mu* opioids and benzodiazepine agonists appear to be additive or less than additive.

In a related study, four other monkeys received 3.2 mg/kg/day of morphine and discriminated between 0.01 mg/kg of naltrexone and saline (e.g., France et al., 1991). Administration of naltrexone (> 0.0032 mg/kg) or termination of morphine treatment occasioned complete (≥90%) naltrexone-lever responding, and this effect was reversed by morphine and other *mu* agonists (e.g., alfentanil). Benzodiazepine receptor agonists neither substituted for naltrexone, attenuated naltrexone-lever responding in morphine-abstinent monkeys, altered the potency of naltrexone in

producing drug-lever responding, nor altered the potency of alfentanil in attenuating drug-lever responding.

Despite reports in the literature of significant pharmacologic interactions between benzodiazepines and *mu* opioids (Antonelli et al., 1986; Rocha et al., 1993), these results fail to demonstrate any enhanced toxicity of opioid/benzodiazepine combinations in terms of effects on ventilation, or to demonstrate significant discriminative (subjective) effects of benzodiazepines when administered either alone or in combination with opioid agonists or antagonists in morphine-treated monkeys. Ongoing studies are investigating potential interactions between *delta* agonists and benzodiazepines.

Another study in this series investigated the effects of several inverse benzodiazepine agonists on monkeys responding under a learning and performance baseline (Auta et al., 1996). In light of several reports indicating that the inverse agonists could enhance the performance of rodents (Chapouthier et al., 1984; Venault et al., 1986) and humans (Duka et al., 1987) in certain behavioral tasks, it was of interest to determine if the same results could be obtained in monkeys responding under a multiple schedule of conditional discriminations. This study was, therefore, designed to directly compare the effects of a full benzodiazepine agonist (alprazolam) with two inverse agonists (β-CCE and FG-7142) and a β-carboline derivative (harmine). Additionally, the benzodiazepine antagonist flumazenil was administered alone and in combination with both types of agonist.

The results obtained from this study demonstrated that β-CCE and FG-7142 produced effects on rates of responding in the learning and performance tasks that were qualitatively similar to each other and to those of alprazolam (the full agonist) and harmine (a drug not thought to interact with benzodiazepine receptors). In contrast, the accuracy data indicated that the inverse agonists were

less disruptive to responding than the full agonist alprazolam, which markedly disrupted learning behavior. Despite these differences in their effects, the data also indicated that the effects of both types of agonist were mediated through the benzodiazepine receptor because the effects of both types of agonist were dose-dependently attenuated by flumazenil. Taken together, this study, involving a complex behavioral procedure and old world monkeys, failed to support previous data obtained with rodents showing that the inverse agonists are capable of enhancing cognitive processes.

The final series of studies conducted during this project period focused on the *delta*-opioid agonist SNC-80, which along with several other *delta* opioids, has been reported to have antinociceptive effects in rodents under a wide range of experimental conditions. SNC-80 is a putative non-peptide, *delta* receptor selective agonist that has been studied under a limited set of conditions *in vitro* and *in vivo* (Bilsky et al., 1995; Calderon et al., 1994). Studies on the effects of SNC-80 in non-human primates have been conducted during the last year and the methods and results of those studies are described in this final report. Briefly, the pharmacological effects of SNC-80 were characterized in rhesus monkeys using a warm-water tail withdrawal procedure to measure antinociception, a head plethysmograph to measure effects on respiration, and a multiple schedule of repeated acquisition and performance to measure effects on complex behavioral processes.

METHODS

Subjects

Adult rhesus monkeys (*Macaca mulatta*) were housed individually and given free access to water. The subjects were fed Purina Monkey Chow and received fresh fruit twice weekly. The subjects that were used to measure complex behavior were maintained at 85% of their free-feeding weights by banana-flavored pellets received during experimental sessions and supplemental feeding in the home cage; all other subjects were maintained at their free-feeding weights. In addition, the subjects involved in drug discrimination studies received 3.2 mg/kg/d (s.c.) of morphine. All subjects, except for two in the antinociception study, had been involved previously in opioid studies.

Apparatus

Antinociception Studies. Primate restraining chairs made of Plexiglass and aluminum piping were used to loosely restrain the subjects at the neck and waist to allow free access to their tails, which hung unimpeded from the bottom of the seat. Thermos bottles were filled with water of different temperatures (40, 50, 55 °C) heated by a hot-water bath. Temperatures were determined to within one degree of the desired temperature using a mercury thermometer. Latency was measured manually by the investigator and recorded using a push-button switch connected to a computer (IBM PCjr).

Ventilation Studies. Subjects were seated in primate restraining chairs made of Lexan, which were located within a sound-attenuating chamber. Alternating layers of Lexan plates (2) and latex collars (2) as well as a foam cushion formed the base of the plethysmograph to form a seal

to minimize gas leakage from the plethysmograph. Air or 5% CO_2 in O_2 was pumped into the plethysmograph at a rate of 10 L/min and removed with a vacuum pump. Changes in air pressure were measured using a pressure transducer and recorded by a microprocessor (Dell Opiplex 433/L). These values were used to calculate f (frequency in resp/min), V_T (tidal volume in L/resp) and V_E (min volume, L/min).

Acquisition and Performance Studies. A removable response panel (69 cm X 22 cm X 47 cm; BRS/LVE, Laurel, MD; model TIP-002) was attached to the side of the home cage (76 cm X 71 cm X 97 cm; Research Equipment Co., Inc., Byran, TX; model LC-1004) during experimental sessions. Three translucent response keys (BRS/LVE, press plate model PPC-012) were located on the response panel 50 cm from the cage floor and 11.5 cm apart. An in-line stimulus projector, mounted behind each of the three keys, projected colors and/or geometric forms onto the keys. Reinforcers were delivered into an aperture (5.5 cm in diameter) located to the right of the rightmost key. Each response panel was connected to a computer and a cumulative recorder located in an adjacent room.

Procedures

Antinociception Studies. A warm-water tail withdrawal procedure was used to measure antinociceptive effects (Dykstra and Woods, 1986). The latency for the subject to remove the tail from the warm water (40, 50, or 55°C) was used as a measure of antinociceptive effect. In order to measure latency, monkeys were placed in chairs and the bottom 10-12 cm of the shaved tail was placed in the water until the subject removed the tail, or until 20s had passed, whichever occurred first. Control (pre-drug) measurements were taken after the animals had been seated in

the chairs for at least 10 min. Drug was administered s.c. on either side of the back or upper arm, alternating sides with each injection. Effective dose ranges and duration of drug effects were determined for each drug using single dose studies with 15 min testing intervals (10 min pretreatment, 5 min period for assessing tail withdrawal lactencies) for a total session time than did not exceed 90 min. Drugs were administered no more than twice weekly, with an intervening period of at least 48 h between tests. Utilizing the dosing information determined with single doses, cumulative dosing studies with 30-min interinjection intervals were used in subsequent experiments. When a maximum effect (20s latency) was obtained in all subjects at 50°C, the session was terminated, except during the determination of the time course of OHM3507 when studies were carried out to 100% maximum possible effect in 55°C water. Control dose-effect curves were conducted using both sterile H_2O and a propylene glycol vehicle. In antagonism studies, a single dose of antagonist (naltrindole or naltrexone) was administered s.c. 10-15 min prior to the initial injection of agonist. Since the duration of the measurable effects of naltrexone (0.01 mg/kg) declines after approximately 2.5 h, sessions with antagonists were limited to 90 min, or a maximum of 5 doses of agonist. Control latencies were determined immediately before the administration of antagonist, and again immediately prior to the first agonist injection.

Respiration studies. These studies are a modification of those previously described by Howell *et al.*, 1988 and France *et al.*, 1990. After placing the head plethysmograph on the Lexan/latex base (described above in **Apparatus**), the primate chair was placed within a sound-attenuating chamber. Experimental sessions consisted of a series of successive,discrete 30-min

cycles, beginning with a saline cycle (as control) followed by 2-6 cycles of either drug or saline. Each cycle was divided into a 23- min exposure to air, followed by a 7-min exposure to 5% CO_2 in O_2. Injections of saline or drug were given s.c. in the back during the first minute of each cycle. Data were recorded for each minute throughout the cycle, but reported as a mean of the last three minutes of exposure to air and 5% CO_2 in O_2. Drugs were administered no more than twice weekly and separated by an intervening period of at least 48 h.

Cumulative dose- response curves were generated (using dosing schedules from antinociception studies), by increasing the amount of drug injected by either half- or quarter-log units in successive cycles. Tests were concluded when subjects attained 50% of control respirations per min (rpm), or for 8 cycles, whichever occurred first. During antagonism studies, a single injection of 0.01 mg/kg of naltrexone was administered one cycle prior to the first dose of agonist.

Drug Discrimination. The procedure for studying drug discrimination in morphine-treated subjects under a fixed-ratio schedule of shock termination has been described elsewhere (France and Gerak, 1994). Briefly, subjects received daily s.c. injections of 3.2 mg/kg/day of morphine 3 hr prior to sessions and discriminated between injections of saline and 0.1 mg/kg of naltrexone. Training sessions consisted of multiple 15-min cycles consisting of a 10 min timeout, during which the chamber was dark and responses had no programmed consequence, followed by a 5-min response period, during which stimulus lights were illuminated and five responses on the appropriate lever (saline- or naltrexone-associated) resulted in the termination of the shock-associated stimulus and postponement of the shock for 30s. Injections (s.c.) were administered during the first minute of the time-out period. Stimuli were terminated after 5 min or 4 shocks,

whichever occurred first. Responses on the incorrect lever reset the response requirement on the correct lever. During saline training sessions, saline injections were administered at the beginning of each cycle; during drug training days, 1-5 saline or sham cycles preceded the administration of naltrexone.

Testing conditions were identical to the training sessions, except that 1) saline was substituted for the daily morphine dose 3 h prior to session, 2) five consecutive responses on either lever resulted in the postponement of shock (no lever was designated as correct) and 3) doses of drug were administered under a cumulative dosing schedule with doses increasing by 1/4 log units. For antagonism studies, naltrexone was administered on the first cycle.

Learning and Performance Studies. A multiple schedule of repeated acquisition and performance of conditional discriminations has been described previously (Moerschbaecher and Thompson, 1983) and served as the base-line procedure. In this procedure the effects of a drug can be evaluated on both the acquisition and performance of a discrimination with a single subject within a single experimental session. In each component of the multiple schedule, the task was to respond on either the right or left key depending upon the stimulus displayed on the center key. Correct responses resulted in progression to the next component of the chain in which a different stimulus was displayed on the center key; incorrect responses resulted in a 5-sec timeout during which reponding had no programmed consequences. Completion of a two-member chain of these discriminations resulted in the delivery of a 500-mg food pellet. During each session a different chain of condtional discriminations was required during one component of a multiple schedule (acquisition/learning component), whereas in the other component the chain of conditional discriminations was the same each session (performance component). The

components alternated after 20 food presentations or 15 min whichever occurred first. A 5-sec timeout, during which all stimuli were off and responses had no programmed consequence, separated consecutive components. Sessions terminated after 200 food presentations or 2 hr, whichever occurred first. Sessions were conducted 5 days per week and always began in the acquisition component. Drug sessions were generally conducted on Tuesdays and Fridays (no more than twice per week), and control (saline) injections on Thursdays. Drugs were administered s.c. in the back 10-15 min prior to the session.

Data Analyses.

Percent of maximum antinociception (%MPE) was calculated in the following manner: % MPE = [(experimental latency - baseline latency) ÷ (20 - baseline latency)]. These values were calculated individually for each subject then averaged among subjects. These mean values (± 1 SEM) were plotted as a function of dose or time. Potency comparisons among drugs were estimated by examining differences in ED_{50}s determined by linear regression (three or more points) or interpolation (2 points). Apparent antagonist affinities (pA_2 and pK_B) were estimated using the methods of Arunlakshana and Schild (1959) as well as the Schild analysis plot with slope constrained to -1 (Tallarida *et al.*, 1979). Physiologic changes in the subjects, e.g. flushing, pupillary dilation, decreased activity, were also noted during antinociception studies, and recorded 5 min prior to each testing interval (in 15 min interval studies) or every 15 min in 30 min interval studies.

Respiratory depression was observed using a comparison of known respiratory indices, f, V_T, and V_e. V_e (minute volume) was calculated by multiplying V_T and f. V_e was plotted as a function of dose of drug for individual subjects in both air and 5% CO_2 in O_2.

Drug discrimination data are presented as the percentage of responding on the drug-associated lever (%NTX) and calculated as: [(number of responses on the naltrexone-associated lever) ÷ (total number of responses)] x 100. These data were plotted (± 1 SEM) as a function of dose. Drugs are considered to substitute for the training drug (naltrexone) when they produce >90% responding on the drug-associated lever.

The effects of drugs on acquisition and performance were determined by calculating the overall response rate (in responses/min), and the percentage of errors ([incorrect/total number of responses] x 100%) for the individual componentsof the multiple schedule. The percentage of errors was calculated for each successive block of 10 food presentations for both saline and drug sessions to measure the within-session learning of the task. Comparisons between drug sessions and the control range of variability in saline sessions, were carried out for each subject. A drug dose was considered to have an effect to the extent that the data fell outside of the control range.

Drugs. Opiods used in these studies were morphine sulfate, fentanyl, naltrexone hydrochloride, and naltrindole hydrochloride (National Institute on Drug Abuse, Rockville, MD), and mirfentanyl and OHM3507 hydrochloride (synthesized by L. L. Brockunier according to Bagley *et al.*, 1989; OHMEDA Inc, Murray Hill, NJ). Drugs were dissolved in sterile 0.9% saline or H_2O (OHM3507). For the antagonism studies with concentrations of drug greater than 1 mg/ml, OHM3507 was dissolved in a vehicle solution of 40% propylene glycol, 50% physiological saline, and 10% ethanol. OHM3507 was made fresh daily as needed.The SNC-80 used in these studies was obtained from Tocris Cookson (St. Louis, MO). It was dissolved in 1 M hydrochloric acid and an equal amount of 1 N and 0.1 N sodium hydroxide, and then further diluted with 0.9% saline.

RESULTS

Major findings during the project period pertaining to the delta opioid receptor are reviewed in this section.

Compound: Ohm3507

Antinociception. Monkeys never removed their tails from 40°C water; control tail-withdrawal latencies for 50 and 50°C ranged from 0.38-0.50s. In time course studies, OHM3507 produced both time- and dose-related increases in antinociception **(FIG 1)**. The onset of action was 15 min, and reached a maximum effect between 30 and 75 min. Full (100%) antinociception occurred in all subjects at a dose of 0.32 mg/kg at 50°C and lasted throughout the entire 90-min testing session; 24 h post-injection, values had returned to baseline measurements (data not included), while maximum antinociception occurred 30 min post-injection in 55°C and declined steadily thereafter. Based upon ED_{50}s obtained from the cumulative dose effect curves, the relative potencies were determined to be: fentanyl (0.12 mg/kg) ≥ OHM3507 (0.14 mg/kg) > morphine (1.77 mg/kg) > mirfentanil (7.44 mg/kg).

Naltrexone produced dose-dependent rightward shifts in the OHM3507 dose-effect curve, and significant increases in the OHM3507 ED_{50}s over a dose range of 0.01 to 0.1 mg/kg of naltrexone **(FIG 2)**. Schild analysis of naltrexone antagonism yielded a slope of -1.25 and produced a pA_2 of 7.8. The pA_2 value with the slope constrained to -1 was 7.81 ± 0.3. Naltrindole (at a dose shown to antagonize agonists acting at the μ receptor [Negus, et al., 1994]), also produced a dose-dependent and rightward parallel shift in the OHM3507 dose effect curve **(FIG 2)**. Calculation of pK_B yielded an affinity estimate of 6.5 for naltrindole.

The propylene glycol vehicle had no effect and redetermination of the OHM3507 dose- effect curve at the end of these studies was not different from the initial OHM3507 curve (data not shown).

Respiration. Morphine produced dose-dependent decreases in VE both air and in 5% CO_2 in O_2. Effective dose ranges for OHM3507 differed widely; however, a decrease was observed in all subjects at doses ranging from 0.1 to 1.0 mg/kg (**FIG 3**). The respiratory depressant effects of OHM 3507 were antagonized by doses of 0.01 mg/kg naltrexone (data not shown).

Drug Discrimination. In morphine-treated monkeys, both the administration of naltrexone in the presence of morphine and the acute deprivation of morphine (27 hr abstinence) produced 100% responding on the drug (naltrexone)-associated lever (data not shown). OHM3507 dose-dependently reversed naltrexone-associated lever responding, with 0.32 mg/kg completely attenuating responding on the naltrexone-associated lever. This effect was dose-dependently antagonized by the administration of naltrexone, and produced a pA_2 of 8.6 ± 0.2 (**FIG 4**).

Learning and performance. In each of the subjects tested OHM3507 produced rate-decreasing effects (**FIG 5, top panels**) in both the learning and performance components at doses greater than 0.1 mg/kg.. No consistent error-increasing effects in either learning or performance were obtained over a dose range of 0.032- 0.1 mg/kg (**FIG 5, Bottom panels**). Pretreatment with 0.032 mg/kg of naltrexone, 40 min prior to the session, produced an antagonism of the rate-decreasing effects of OHM3507. A greater than 10-fold shift to the right in the OHM3507 dose response curve was obtained with this dose of naltrexone.

Other Effects of OHM3507. Several behavioral effects, e.g. mydriasis, sedation, flushing of the face, salivation, were observed in most subjects at approximately one-tenth of the doses (0.32 mg/kg) necessary to produce 100% antinociception. There was some indication in the acquisition and performance studies that OHM 3507 continues to exert some rate-decreasing effects 24 hr or more post-injection. These effects seemed to diminish in intensity with continued exposure to the drug in the antinociception and respiration studies, but not in the study of complex behavioral processes.

Compound: SNC-80

Antinociception Studies. SNC-80 was studied in 4 rhesus monkeys, up to a dose of 3.2 mg/kg s.c., and failed to have any effect on the latency for monkeys to remove their tails from warm water (see **Methods** for details). These negative data in rhesus monkeys, though inconsistent with results obtained in other species (e.g., Bilsky et al., 1995), are fully consistent with the lack of antinociceptive effect obtained with another purported *delta* selective agonist, BW373U86, under the same conditions in monkeys (unpublished observation). In contrast, morphine increased tail withdrawal latency in a dose-related manner, with similar results obtained when the inter-injection interval was either 15 or 30 minutes. Thus, neither SNC-80 nor BW373U86 have antinociceptive effects in rhesus monkeys, indicating that there might be significant differences among species in the analgesic effectiveness of *delta* opioids; in contrast, morphine and other *mu* opioids have robust antinociceptive effects in many species and under a wide range of experimental conditions.

Ventilation Studies. In these studies, SNC-80 decreased the frequency and volume of ventilation in a dose-related manner (FIGS 6 and 7). Figure 6 shows the time course of effects

for 0.1-1.0 mg/kg of SNC-80 for monkey Maus breathing air (left panels) or 5% CO_2 in air (right panels). There were dose-related decreases in all measures of ventilation for both conditions with maximal effects obtained within 30 minutes of s.c. injection. In this monkey, a dose of 1.0 mg/kg of SNC-80 (a dose that has no antinociceptive effect in this monkey) decreased minute volume to less than 50% of control (open circle, upper left panel) in air. The effects of SNC-80 on ventilation in air were larger than the effects on ventilation in 5% CO_2. Typically, the largest effects on ventilation for *mu* opioids was obtained in the presence of CO_2. Whether these limited results with SNC-80 are indicative of a significant difference between *delta* and *mu* opioids with regard to effects on ventilation under strained conditions (e.g., CO_2) or whether this is an anomalous finding from a monkey that shows a relatively modest hyperventilation to 5% CO_2 has yet to be established.

Figure 7 shows the dose-relatedness of respiratory depression produced by SNC-80 in monkey Maus plotting the maximum observed effect as a function of dose. One particularly intriguing feature of these data is the potent respiratory depressant effect of SNC-80 that occurs in the absence of antinociceptive effect. This profile of effects is opposite to that which has been reported in other species and opposite to the presumed beneficial aspects of *delta* receptor agonists (i.e., robust antinociceptive effects in the absence of significant respiratory depression). Ongoing studies are assessing the nature of the respiratory depression produced by SNC-80 using selective opioid antagonists to first determine the opioid receptor type that mediates these unexpected effects.

Acquisition and Performance Studies. Under control conditions, the accuracy and rate of responding in both components of the multiple schedule remained stable. Although mean overall

response rates were generally higher in performance, response rates in both components were consistent from session. Accuracy, as indicated by the percentage of errors in each component, also remained stable from session to session. Moreover, the response patterns for each monkey in acquisition were characterized by distinct decreases in the numbers of errors and an increase in consecutive errorless completions of the discrimination indicating a steady state of learning in terms of within-session error reduction. This response pattern at the start of the session in acquisition under control conditions also accounted for the fact that mean percent errors in acquisition were typically larger than mean percent errors in performance.

As shown in Fig. 8, SNC-80 produced dose-dependent rate-decreasing effects in both components of the multiple schedule in all three subjects. In one subject, subject A, these rate-decreasing effects tended to occur in the acquisition component before they occurred in the performance component. This can clearly be seen at the 0.32 and 0.56 mg/kg doses of SNC-80 in this subject. More substantial differences in the effects of SNC-80 were observed on the accuracy of responding. In all three subjects, for example, SNC-80 produced dose-dependent increases in percent errors in acquisition, but produced little or no effect on percent errors in performance across these same dose range. It should be noted, however, that these error-increasing effects in acquisition generally occurred at doses of SNC-80 that also substantially decreased the overall rates of responding in each subject. The differential effects obtained on the accuracy of responding are evident at the 1 mg/kg dose in monkey A, the 3.2 mg/kg dose in monkey TN, and the 1 - 5.6 mg/kg doses in monkey NC.

The effects of SNC-80 on the within-session patterns of responding are shown for one subject (subject NC) in Fig. 9. As depicted by the record in the top row, under control conditions, errors

in acquisition generally decreased to near zero levels shortly after the start of the session and remained that way for the rest of the session. This characteristic decrease in errors and increase in error-less responding as the session progressed indicates the point at which the subject was considered to have acquired the correct sequence of responses. This pattern of errors in acquisition was in direct contrast to the pattern of errors in performance where errors were consistently near zero during the first cycle and remained that way throughout the session.

When 1 mg/kg of SNC-80 was administered to this subject, the pattern of responding in both components was substantially altered. In comparison to the control record, for example, there was very little responding during the first acquisition component and acquisition of the sequence for that session does not occur until the second acquisition component. Moreover, a small increase in errors is evident for responding in acquisition. The rather selective nature of this effect is also demonstrated in this record by the pattern of responding in performance where no error-increasing effect occurs even though the overall rate of responding is clearly decreased in this component. One might be tempted to speculate that this absence of an error-increasing effect in performance occurs because the drug effect is waning after the first acquisition component. However, this would not explain the small increase in errors that occurs at the end of the first acquisition component and at the beginning of the second acquisition component.

CONCLUSIONS AND FUTURE PLANS

The behavioral and pharmacologic profile of OHM3507 in these studies demonstrates that this compound has both high selectivity and efficacy at the μ opioid receptor in rhesus monkeys, producing effects similar to fentanyl. OHM3507 was evaluated previously in rats, rabbits and mice (Bagley et al., 1989, Wynn et al., 1991) However, the effects of OHM3507 in rodents are not consistent with those seen in these studies in rhesus monkeys.

OHM3507 has a high degree of efficacy at the μ-opioid receptor, producing antinociceptive effects as well as decreasing respiration in both air and 5% CO_2 in O_2. These effects were antagonized by the administration of the opioid antagonist naltrexone, producing rightward shifts in the agonist dose-response curves.

Another measure of the strong μ opioid actions of OHM3507 is its ability to reverse withdrawal in morphine-dependent subjects. Withdrawal can reliably be precipitated in chronically-treated subjects by either the administration of naltrexone, an opioid antagonist, or the deprivation of the daily dose of morphine (France and Woods, 1989; France and Gerak, 1994). In morphine-treated subjects discriminating between injections of saline and naltrexone and acutely deprived of morphine, responding on the naltrexone-associated lever was fully attenuated by OHM3507. This attenuation is similar to that seen with morphine, and other μ-selective opioid agonists.

In addition to some of the observable signs associated with opioid agonist administration (i.e. mydriasis, sedation), OHM3507 also affected complex behavior in a manner similar to morphine (Moerschbaecher and Thompson, 1983). In both learning and performance, the percent of errors produced did not change substantially with the administration of OHM3507 until high doses ,

where rates of responding decreased greatly. Thus, OHM3507 does not produce selective effects on learning and memory doses lower than those that affect the mechanical performance of these tasks.

Opioid antagonists have been used extensively to differentiate receptor mechanisms through their varying degrees of selectivity and affinity. Naltrexone dose-dependently shifted the OHM3507 dose-effect curve to the right and the pA2s and pK_Bs determined for OHM3507 lie within the dose range established for naltrexone in combination with other μ-selective opioid agonists (France and Gerak, 1994). Additionally, antagonism with naltrindole, at doses that would affect both μ and κ receptors produced a rightward shift in the dose-effect curve; the affinity estimate falls within the range estimates for μ-receptor mediation (Negus et al., 1993).

The purpose of this study was to explore the pharmacological profile of OHM3507, and its potential use as a compound with abilities to produce antinociception, but with reduced adverse effects, based on its profile in other species. However, one characteristic that seems to be emerging about this class of like compounds is the substantial differences seen in physiological and behavioral effects produced with these compounds among species (France et al, 1991; France et al., 1994, France et al, in press). It may be necessary, by using well-established procedures and a variety of species to produce a more accurate pharmacological profile of these compounds in order to better and more accurately predict the possible effects of these drugs in humans, as well as explore the differences that species play in producing the pharmacological effects.

We have previously reported on our studies of BW373U86 using these same methods. While this compound clearly had greater δ-receptor activity than OHM3507 it was disappointing in terms of its overall pharmacological profile. It exhibited relatively poor systemic activity and significant behavioral toxicity including convulsions and barrel rolling (Comer et al., 1993; Dykstra et al., 1993; Pakarinen et al., 1993). Despite these adverse effects BW373U86 has proven to be an effective lead compound for the development of nonpeptidic δ-opioid agonists. Bilsky et al. (1995) have reported on the pharmacology of modified enantiomer of BW373U86, SNC 80. They profiled the pharmacology of this compound both in vitro and in vivo. Their data indicated that it was systemically active and highly selective for the δ-opioid receptor. We focused on this compound in our last major series of studies during the project period.

A range of doses of SNC-80 have been evaluated in four monkeys. It is worth noting that the response of these four rhesus monkeys to the effects of SNC-80 on ventilation has been variable: two of four monkeys are quite sensitive to SNC-80 and in these monkeys the respiratory depressant effects are robust; two other monkeys are less sensitive to SNC-80 with increases in ventilation sometimes observed after the administration of large doses (e.g., 5.6 mg/kg). It is not clear why these monkeys differ so dramatically in their response to SNC-80 and we do not yet know whether the respiratory stimulation that is observed in some monkeys is mediated by opioid receptors. Future studies will focus on potential interactions between SNC-80 and *mu* opioids, since there have been several reports suggesting that *delta* agonists can enhance the antinociceptive potency and effectiveness of *mu* opioids (e.g., Jiang et al., 1990; Noble et al., 1994). It will be especially interesting to see whether a *delta* agonist which has no

antinociceptive effects itself can modify the antinociceptive effects of morphine (*mu* agonist) and whether there are interactions between the apparently similar respiratory depressant effects of these two classes of compounds.

Future behavioral studies in monkeys will determined if the effects produced by *delta* agonists on acquisition behavior or learning were mediated directly by *delta* receptors or are mediated via an interaction between *delta* receptors and *mu* receptors. Just as the effects of SNC-80 on respiration appear to be similar to the effects of *mu* opioid agonists, the effects of SNC-80 on learning are similar to those observed previously for many *mu* opioid agonists (Moerschbaecher and Thompson, 1983, Moerschbaecher et al., 1983). Morevover, there are reports in the literature that have suggested a functional coupling between *mu* and *delta* receptors (Noble et al., 1994). Regarding this question, we examined the mechanism for the observed effects of SNC-80 on learning by administering SNC-80 in combination with the *mu* receptor antagonist naltrexone and by administering SNC-80 in combination with the *delta* receptor antagonist naltrindole; while making sure that the doses of naltrindole were low enough to maintain their *delta*-selective activity.

Two other observations from the present studies with *delta* opioid agonists are noteworthy. The first observation, as discussed briefly above, concerns the differential sensitivity shown between subjects for the effects of SNC-80 and other delta ligands on ventilation and complex operant processes. While the second observation concerns the distinct species differences noted between rodents and rhesus monkeys for these ligands. Although the data are not shown in this report, SNC-80 was administered to a fourth subject responding under the multiple schedule of acquisition and performance. However, in this subject, no effect was observed on either response

rate or accuracy of responding up to a dose of 3.2 mg/kg. The fact that there was no effect in this subject at a relatively high dose, and that there were dramatic differences in the response of two monkeys to SNC in the ventilation study, raises additional questions about the potential therapeutic value of this ligand. Furthermore, our results in rhesus monkeys suggests that rats may not be the most appropriate animal model for examining the effects of opioid receptor agonists. Not only have our results with SNC-80 been in direct contrast with those reported for rats, but the data reported for OHM3507 also indicated that the effects found in rhesus monkeys on a variety of measures (e.g., ventilation, nociception, drug discrimination and complex learning processes) were not consistent with those seen in rats. Thus, as has been demonstrated for the OHM series of drugs in the 4-heteroanilido-piperidine class (France et al., 1991,1995c; Ahn et al., in press), there seems to be an emerging characteristic for many of the delta receptor ligands indicating substantial differences in physiological and behavioral effects produced among species, and possibly within species.

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FIGURE LEGENDS

Figure 1. Time course studies for the antinociceptive effects of OHM3507 using 50°C (upper panel) and 55°C (lower panel) water. N = 4 for all studies, except that of 0.01 mg/kg OHM3507 where N = 3. Ordinates: percent of maximum possible effect (%MPE) ± 1SEM; abscissae: dose in mg/kg body weight.

Figure 2. Antagonism of OHM3507 by naltrexone (left) and naltrindole (right) using a cumulative dosing procedure. Subjects received an injection of naltrexone 10 min before sessions. N = 4 for all groups. See Figure 1 for other details. Lower panel : Schild plot of same antinociception data presented in the upper panel. Ordinate: log(dose ratio - 1); abscissa: - log(dose of naltrexone in moles/kg).

Figure 3. Dose effect curves of morphine, and OHM3507 on respiration in air and 5% CO_2 using a cumulative-dosing schedule. Values (in air) were taken after injection with saline one session prior to initial injection with drug, and used as baseline. Morphine and OHM3507 results are expressed in terms of V_E (% control) for both air and 5% CO_2 in O_2. Abscissae: dose in mg/kg of body weight; C = control (no drug).

Figure 4. Discriminative stimulus effects of OHM3507 in subjects treated with 3.2 mg/kg/day of morphine and discriminating between injections of saline and naltrexone. For these studies, monkeys received saline, rather than morphine, 3 hrs prior to session. OHM3507 was studied

alone (closed circles) as well as in combination with several doses of naltrexone. Ordinate: percent responding on drug-associated lever (%DR); abscissa: dose in mg/kg of body weight.

Figure 5. The effects of OHM3507 on acquisition and performance. Subjects were treated with a dose of drug or vehicle 10 min prior to experimental session. The results of each individual subject are presented (left-Co, middle-B, right-P). Drug data in both the performance (filled circles) and acquisition (open circles) components represent the mean and range of at least two determinations in each subject. Th ecombined effects of OHM3507 and 0.032 mg/kg naltrexone are indicated by triangles (open = acquisition component; closed = performance component). Ordinates: rate in responses/min (upper panels) and percent errors (lower panels); abscissae: dose in mg/kg of body weight.

Figure 6. Time course studies of SNC-80 on respiration in air and 5% CO_2 in air in subject Maus at doses of 0.1, 0.32 and 1 mg/kg. SNC-80 results (ordinates) are expressed in terms of V_E (%control), V_T (%control) and f (%control) for both air and 5% CO_2 in O_2. Abscissa: time in minutes. S=effects of saline.

Figure 7. Dose effect curves of SNC-80 on respiration in air in subject Maus using a cumulative-dosing schedule. Values (in air) were taken after injection with saline one session prior to the initial injection with drug, and used as baseline. See figure 2 for other details.

Figure 8. Effects of SNC-80 on overall response rates and percent errors in the acquisition and performance components of a multiple schedule in subjects A, TN and NC. Subjects were treated 30 min prior to the start of the experimental session with a dose of drug or vehicle. The unconnected data points and vertical lines to the left of the dose-effect curves at V indicate the mean and range of 8 to 20 vehicle or saline control sessions for that subject. Data points with vertical lines in the dose-effect curves for both acquisition (open circles) and performance (filled circles) represent the mean and range of at least two determinations of that dosage in each subject. Data points (open or filled) without vertical lines in the curves indicate either a single determination of that dosage or an instance in which the range is encompassed by the data point. Ordinates: rate in responses/min (upper panels) and percent errors (lower panels); abscissae: dose in mg/kg of body weight.

Figure 9. Cumulative response records for monkey NC showing the within-session effects produced in the acquisition (A) and performance (P) components of the multiple schedule following either saline or a 1 mg/kg dosage of SNC-80. Each record shows all but a few minutes of the experimental session (i.e., approximately 10 minutes). The response pen (upper pen in each record) stepped upward with each correct response and deflected downward each time the five-response sequence was completed. Errors in both components are indicated by deflections of the event pen (lower pen in each record). A change in components of the multiple schedule, which occurred after either 20 min or 15 reinforcements, reset the response (stepping) pen.

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Personnel supported during the project period are listed below by year.

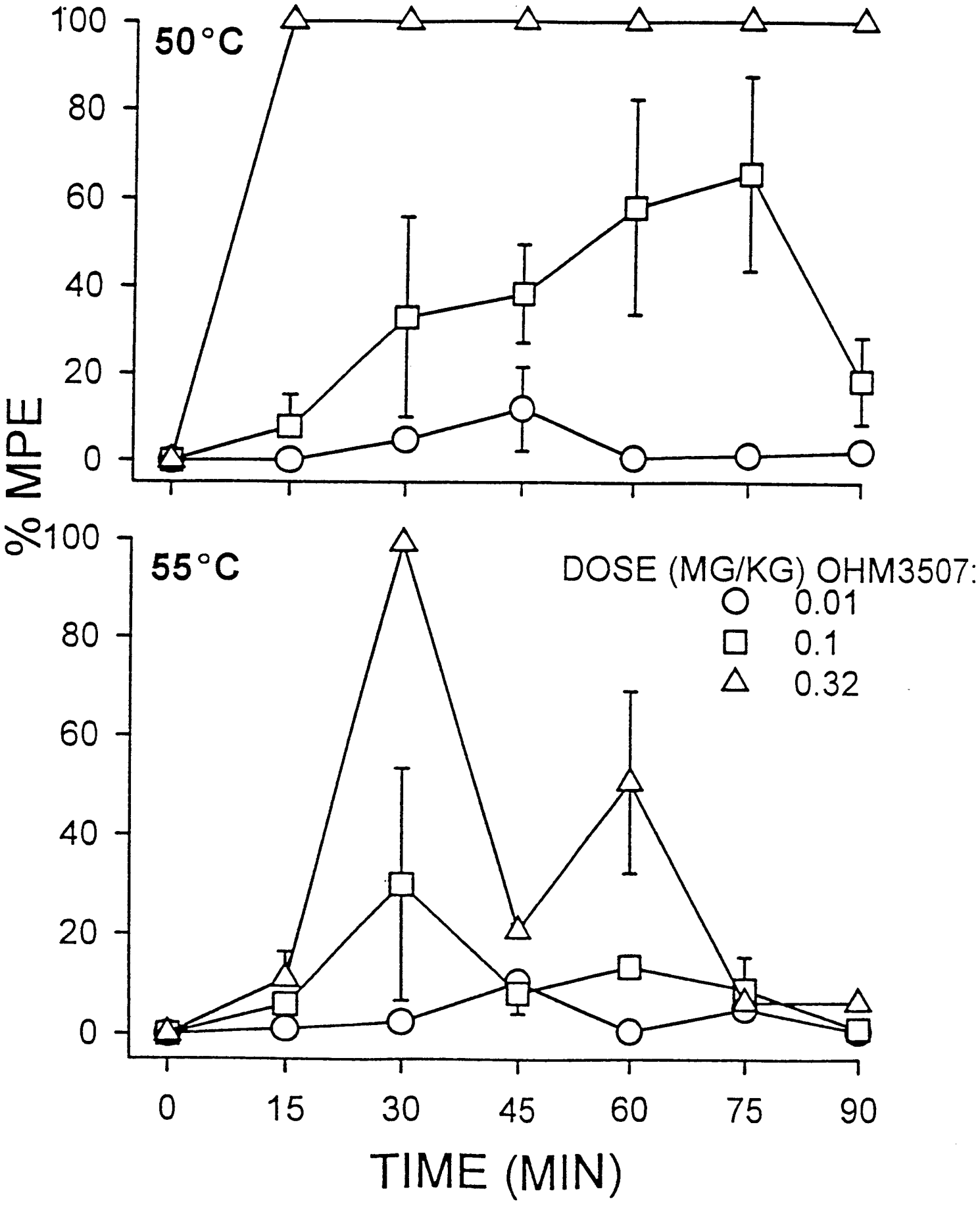
94-95
Auta, James
Fortier, Renee
Landers, Laura
Mains, John
Winsauer, Peter

95-96
Landers, Laura
Winsauer, Peter

96-97
Landers, Laura
Mitchell, Janel
Winsauer, Peter

97-98
Chu, Sally
Winsauer, Peter

APPENDICES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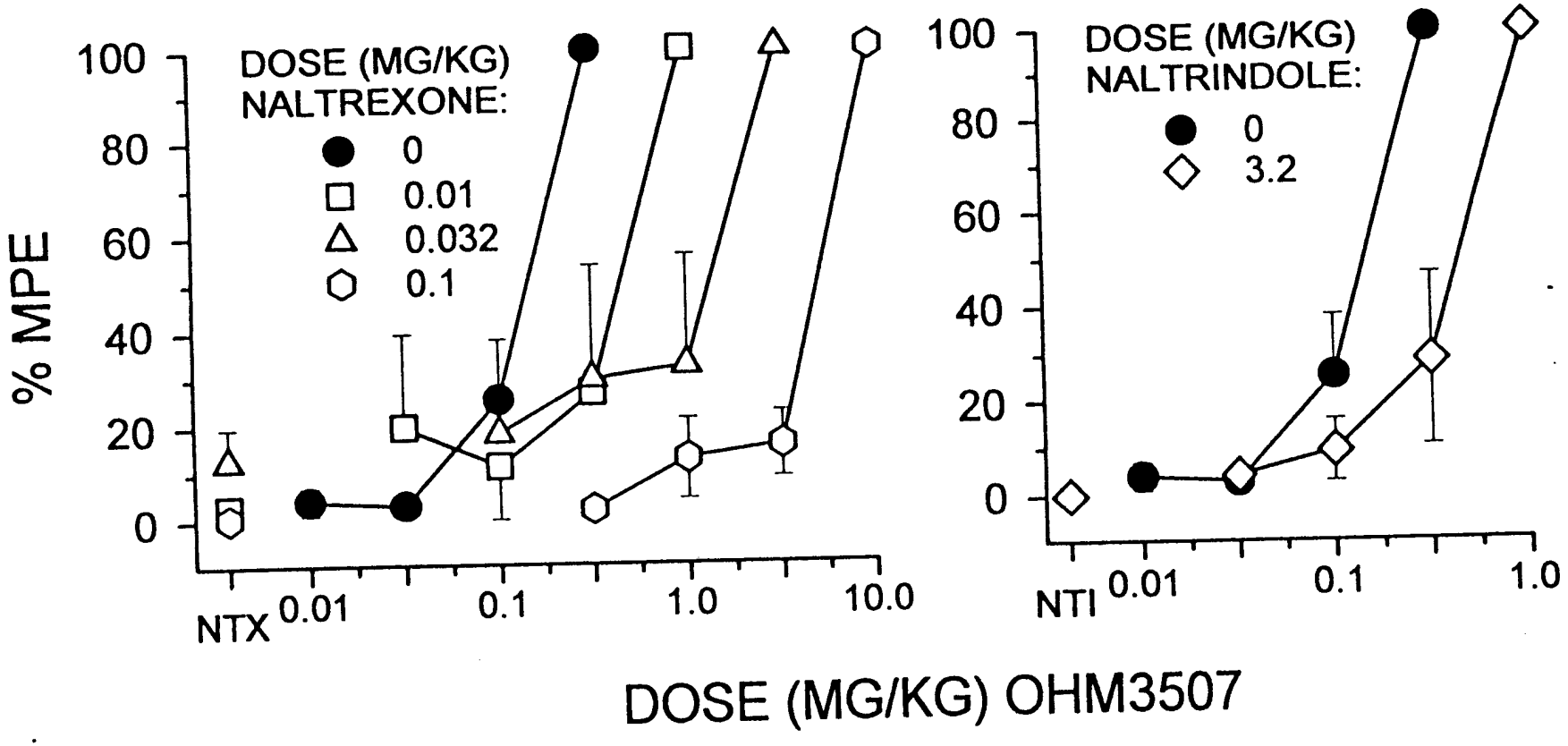
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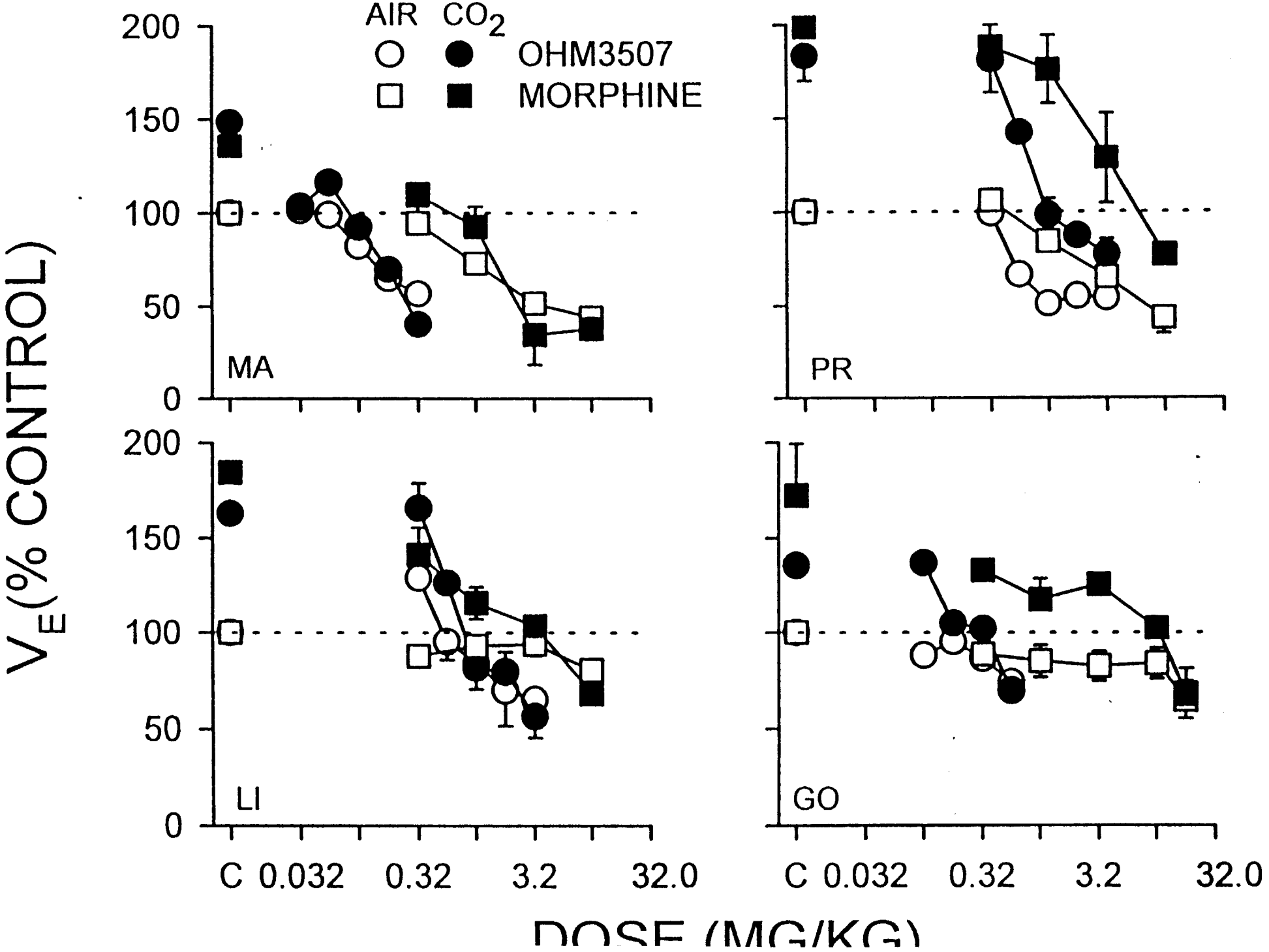
1.0
10.0
DOSE (MG/KG)
NALTRINDOLE:
0
3.2
NTI
0.01
0.1
1.0
DOSE (MG/KG) OHM3507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OHM3507
MORPHINE
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V_E(% CONTROL)
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100
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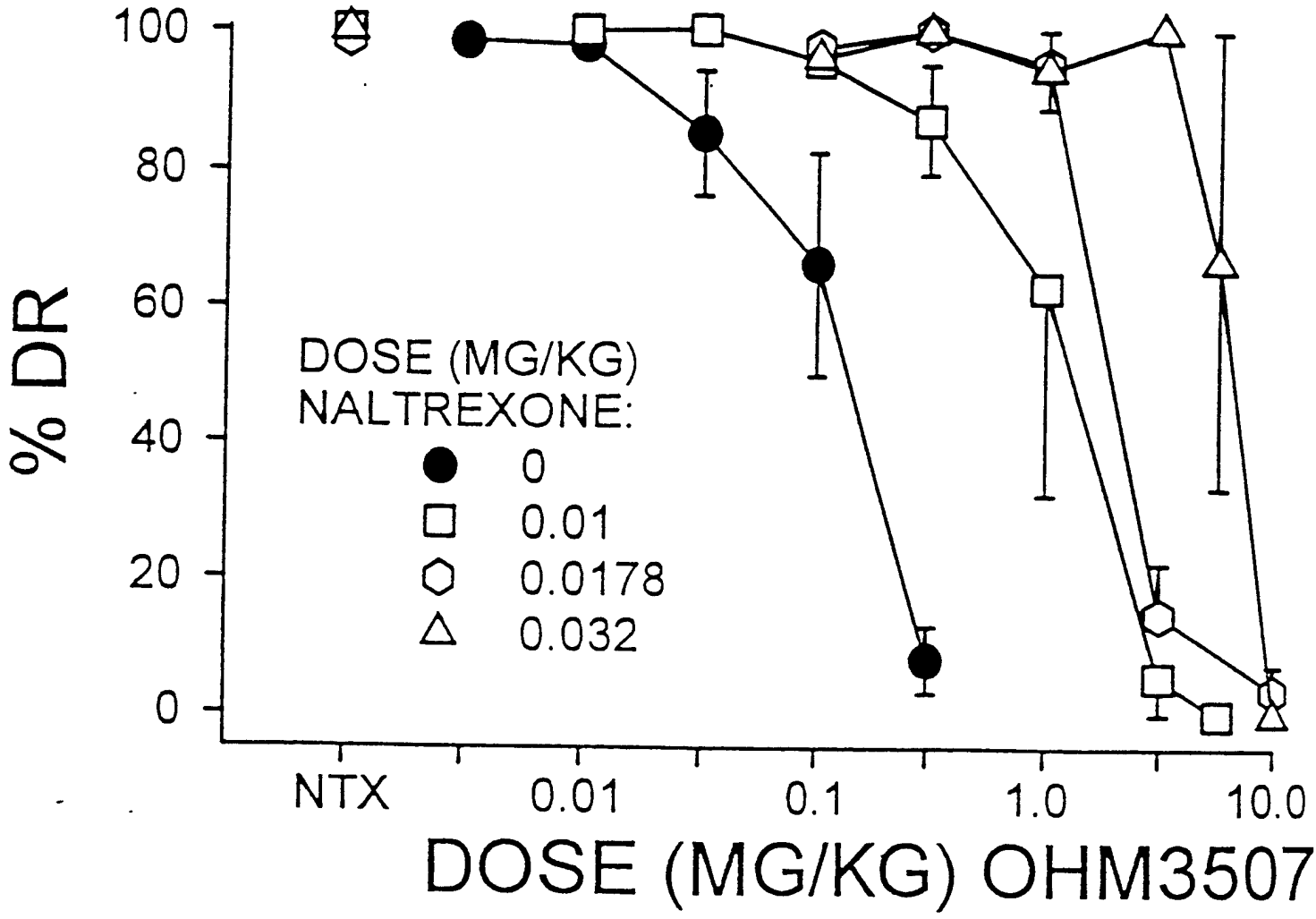
DOSE (MG/KG)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80
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DOSE (MG/KG)
NALTREXONE:
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0.01
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0.1
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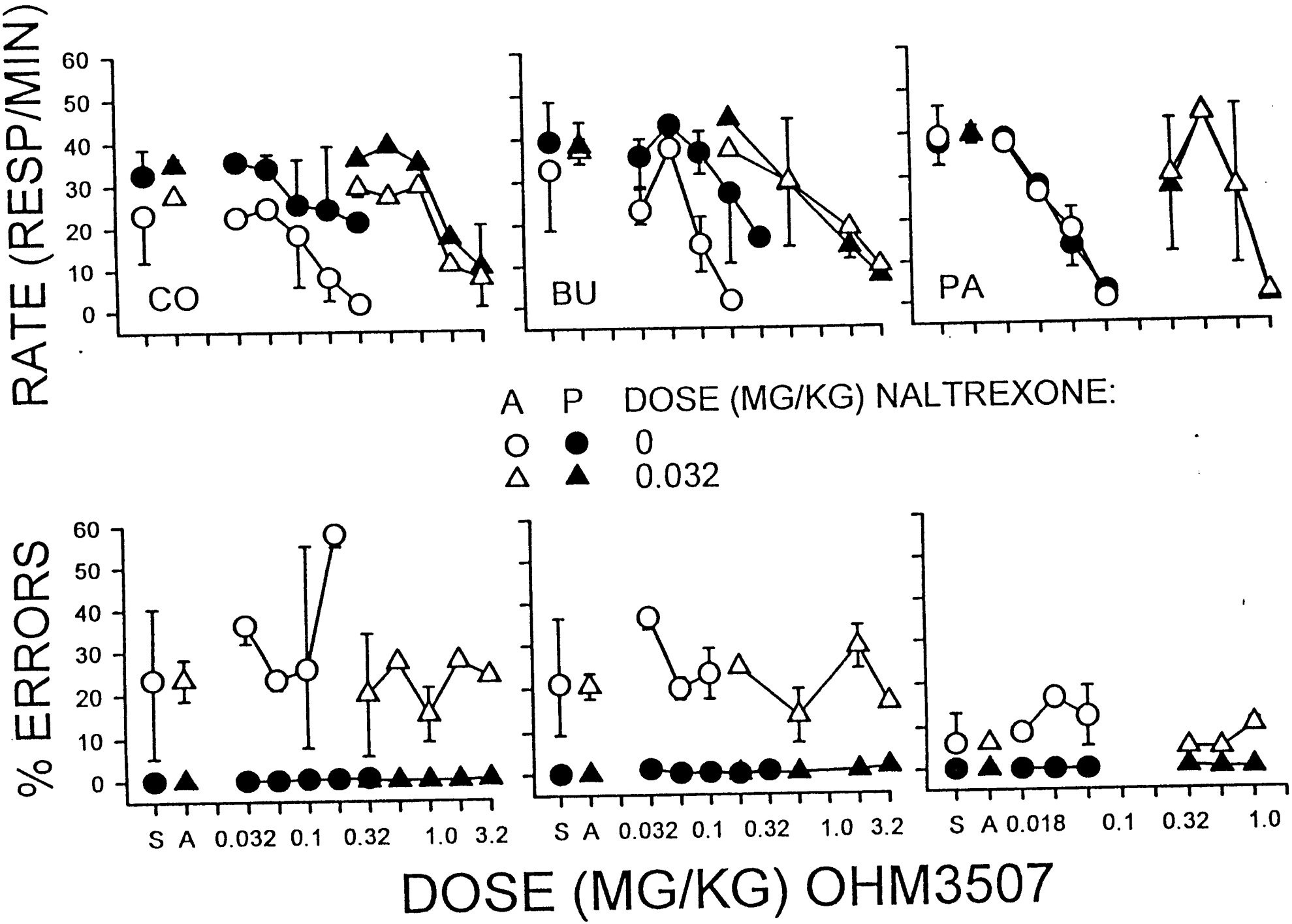
DOSE (MG/KG) OHM3507


RATE (RESP/MIN)
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A P DOSE (MG/KG) NALTREXONE:
0
0.032
% ERRORS
S A 0.032 0.1 0.32 1.0 3.2
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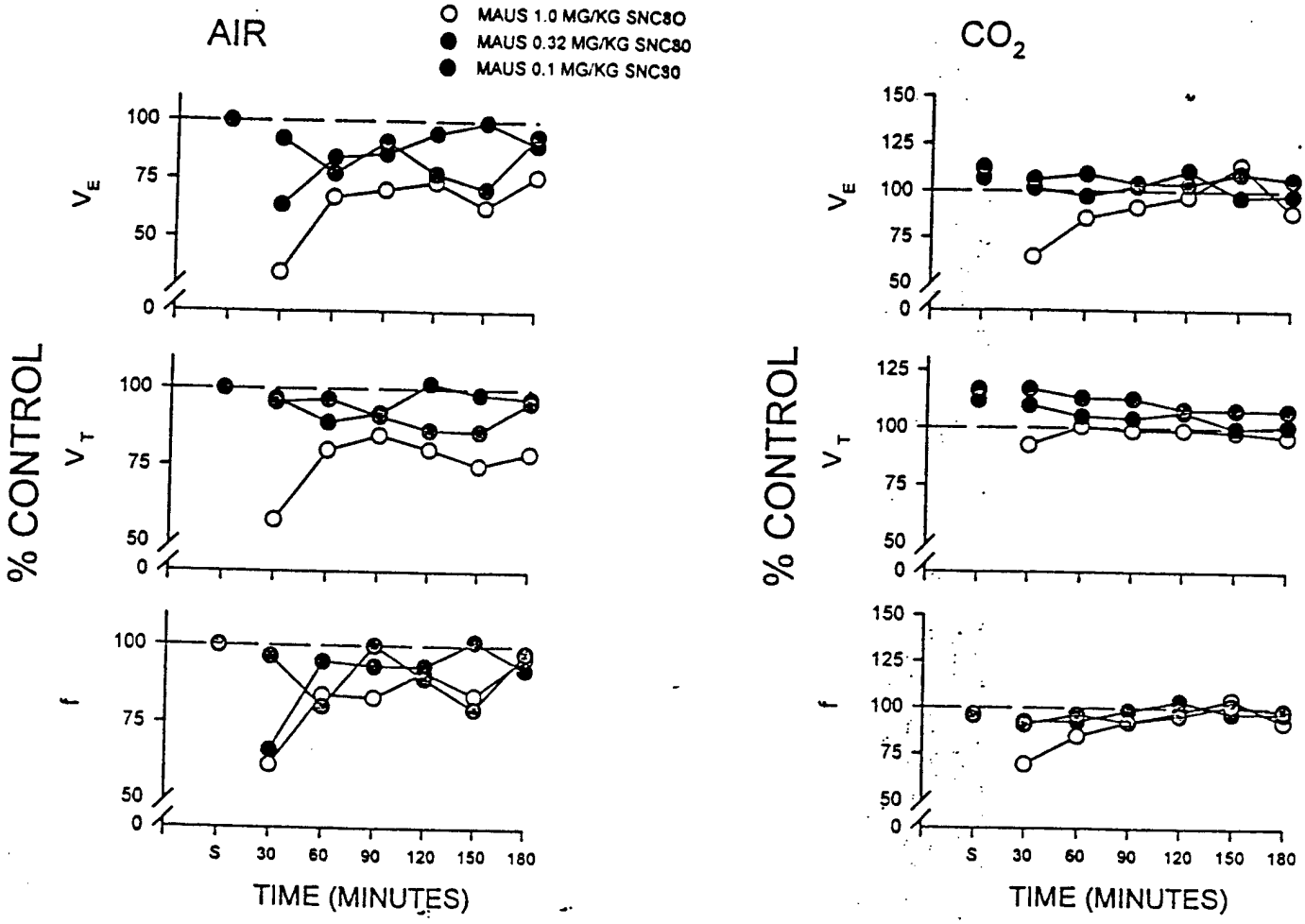
S A 0.018 0.1 0.32 1.0
DOSE (MG/KG) OHM3507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MAUS 1.0 MG/KG SNC80
MAUS 0.32 MG/KG SNC80
MAUS 0.1 MG/KG SNC80
% CONTROL
V_E
V_T
f
100
75
50
0
S
30
60
90
120
150
180
TIME (MINUTES)
CO_2
% CONTROL
V_E
V_T
f
150
125
100
75
50
0
S
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120
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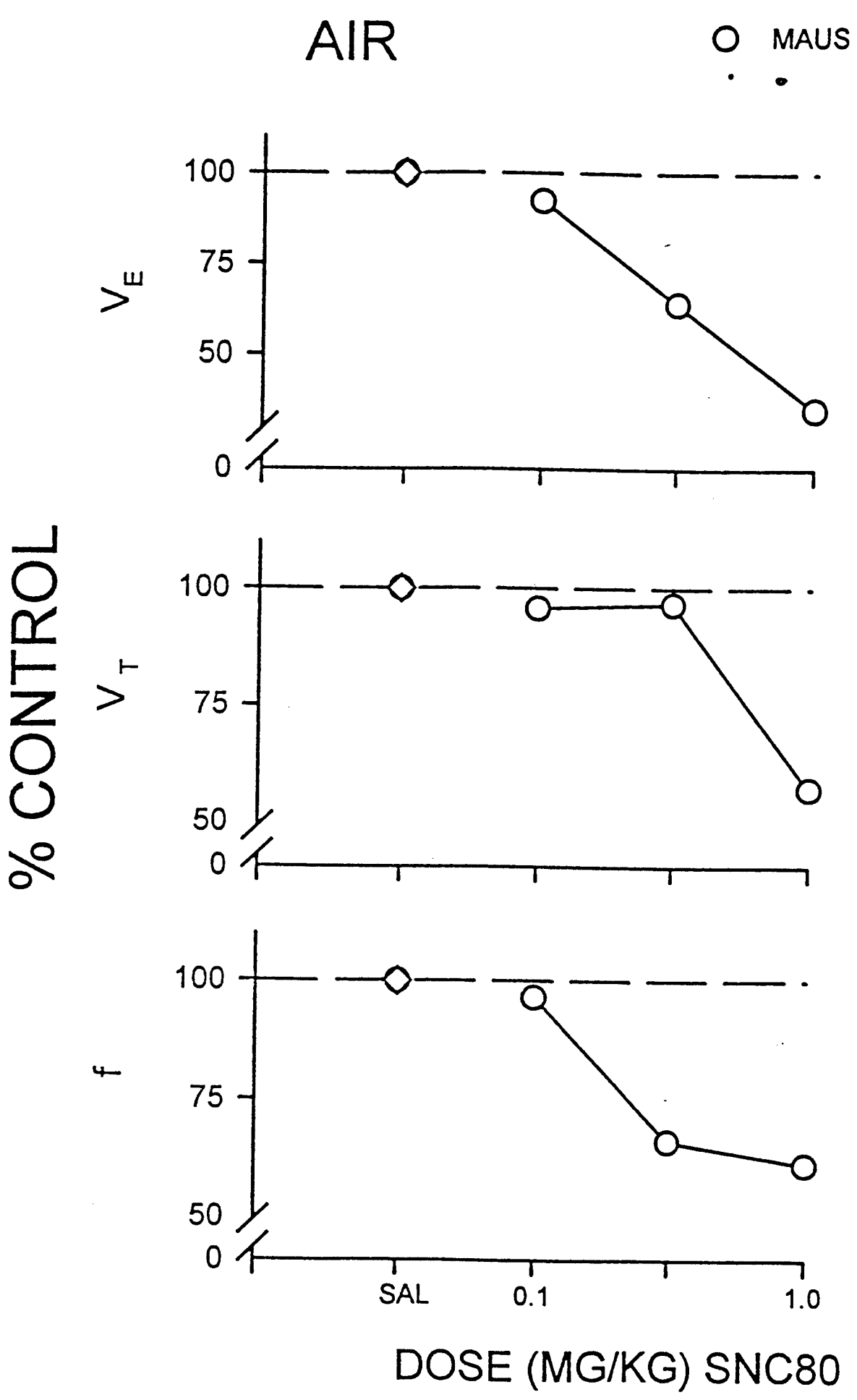
180
TIME (MINUTES)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MAUS
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V_T
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SAL
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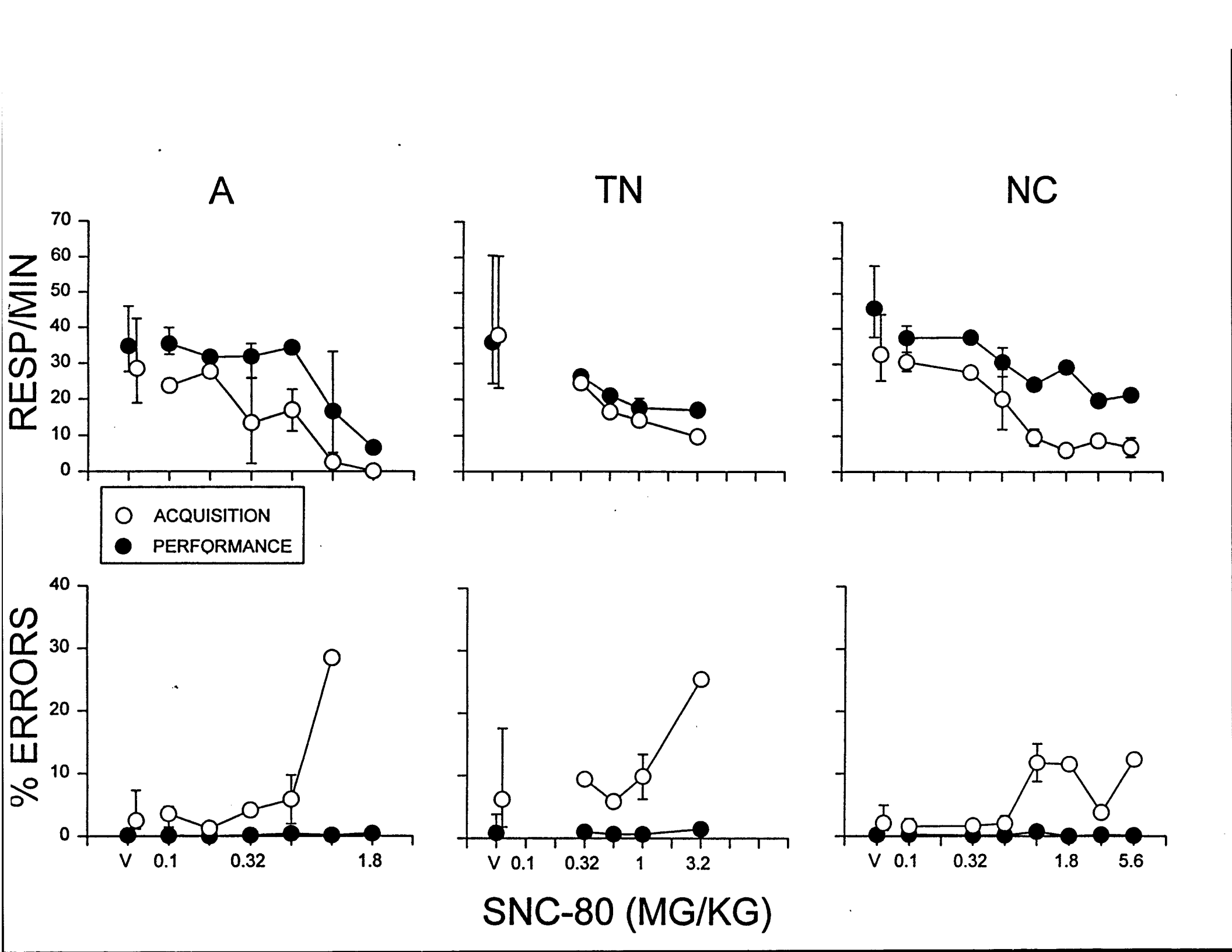
1.0
DOSE (MG/KG) SNC80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RESP/MIN
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50
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ACQUISITION
PERFORMANCE
% ERRORS
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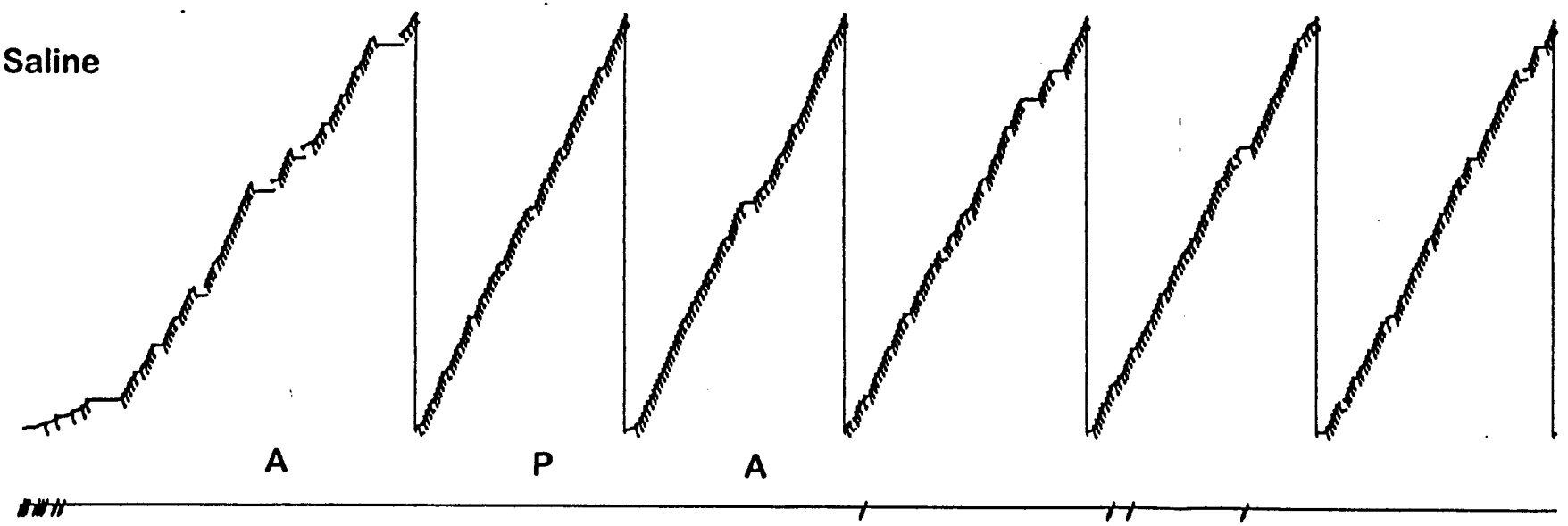
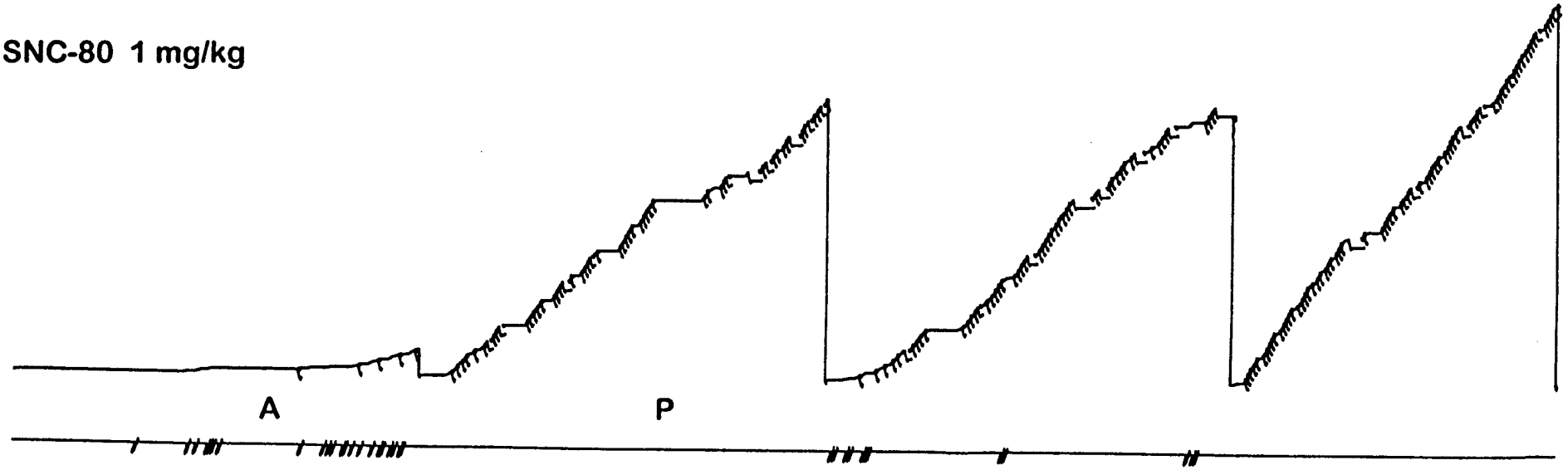
V
0.1
0.32
1.8
V
0.1
0.32
1
3.2
V
0.1
0.32
1.8
5.6
SNC-80 (MG/KG)

MONKEY NC

Saline



SNC-80 1 mg/kg



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Behavioral Effects and Binding Affinities of the Fentanyl Derivative OHM35071

C. P. FRANCE,* S. C. AHN,* L. L. BROCKUNIER,† J. R. BAGLEY,† M. R. BRANDT,* P. J. WINSAUER* and J. M. MOERSCHBAECHER*

*Department of Pharmacology and Experimental Therapeutics, Louisiana State University Medical Center, 1100 Florida Avenue, New Orleans, LA 70119, and †Ohmeda, Inc., The BOC Group Technical Center, 100 Mountain Avenue, Murray Hill, NJ 07974

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FRANCE, C. P., S. C. AHN, L. L. BROCKUNIER, J. R. BAGLEY, M. R. BRANDT, P. J. WINSAUER AND J. M. MOERSCHBAECHER. *Behavioral effects and binding affinities of the fentanyl derivative OHM3507.* PHARMACOL BIOCHEM BEHAV 59(2) 000–000, 1997.—Several fentanyl derivatives have been reported to have novel pharmacologies that might be exploited for developing alternate approaches to the treatment of pain. The purpose of the current series of studies was to evaluate OHM3507, a novel fentanyl derivative reported to have an unusual pharmacological profile in nonprimate species. Similar to several other fentanyl derivatives with clinical potential, OHM3507 had the highest affinity (IC_{50} = 10 nM) for – [³H]D-Ala²,N-Me-Phe⁴, Gly⁵-OH–labeled) receptors with 6- and 176-fold lower affinity for δ ([³H]D-Pen²-D-Pen⁵–labeled), and κ [³H]ethylketocyclazocine–labeled) receptors, respectively. In rhesus monkeys, OHM3507 shared discriminative stimulus effects with morphine, increased tail-withdrawal latencies in a warm-water procedure of antinociception, decreased ventilation in monkeys breathing normal air or 5% CO_2, and failed to modify accuracy on acquisition and performance tasks up to doses that decreased rates of food-maintained responding. The opioid antagonists naltrexone and naltrindole antagonized the behavioral effects of OHM3507 in a manner that was consistent with μ-receptor mediation. Although OHM3507 appeared to have low efficacy opioid actions in nonprimate species, results from the current studies clearly show this compound to have strong, fentanyl-like μ agonist actions in rhesus monkeys. These results provide another example of the sometimes poor predictability in the behavioral pharmacology of fentanyl derivatives among species, in this case between monkeys and rats, mice and rabbits, and demonstrates the need for evaluating new drugs under a broad range of conditions to increase the probability of identifying novel compounds that can be used to treat pain. © 1997 Elsevier Science Inc.

Acquisition and performance Antinociception Drug discrimination Fentanyl Mirfentanil OHM3507 Opioids Rhesus monkey

MU opioid agonists continue to be the drugs of choice in the treatment of moderate to severe pain, despite the well-established toxicity and abuse liability of most compounds in this pharmacological class. Notwithstanding the need for strong analgesics that have reduced abuse liability and reduced toxicity, there has been relatively little success in the effort to develop alternate pharmacological approaches to the treatment of pain. Thus, virtually all opioid agonists that are effective in treating moderate to severe pain also have high abuse liability, produce physical dependence, and decrease ventilatory function.

Fentanyl (Fig. 1) is a morphine-like (i.e., μ-receptor selective) opioid agonist that is used widely in anesthesia and, to a lesser extent, to treat pain. Like morphine, fentanyl has a very high abuse liability, produces physical dependence, and decreases ventilatory function (1,12,13). Recently, several 4-heteroanilido-piperidine derivatives of fentanyl have been shown to have robust antinociceptive effects in nonhuman primates and not to have some of the other undesirable effects that are typical of morphine-like opioids (3,6,8). One compound in this chemical series, mirfentanil, has an especially interesting profile of effects. For example, mirfentanil has sufficiently low efficacy at μ opioid receptors that, like naltrexone, it precipitates withdrawal in morphine-dependent subjects (8); it also has antinociceptive effects and respiratory-depressant effects in rodents while reversing the antinociceptive effects of morphine in rabbits (19) and rats (3). In rhesus monkeys, the antinociceptive effects of mirfentanil are not mediated by opioid receptors and, compared to other opioid agonists, the effects

Requests for reprints should be addressed to C. P. France, Department of Pharmacology, Louisiana State University Medical Center, 1901 Perdido, New Orleans, LA 70112-1393.

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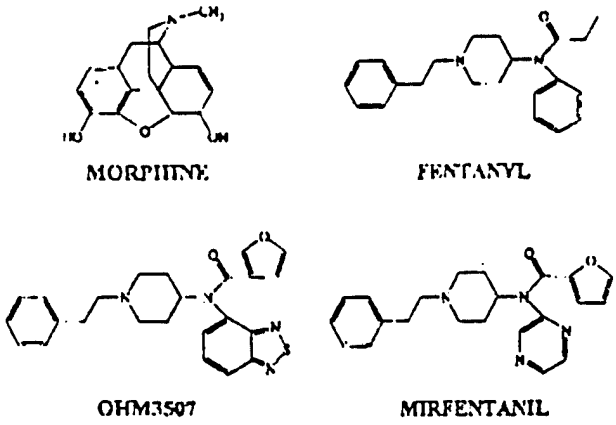

FIG. 1. Structures of morphine, fentanyl, OHM3507, and mirfentanil.

of mirfentanil of ventilatory function are very modest. Another compound in this series, OHM3295, also has nonopioid antinociceptive effects in nonhuman primates as well as low-efficacy μ agonist actions (6). These antinociceptive effects of OHM3295 and mirfentanil are not blocked by naltrexone or other opioid antagonists, suggesting that these effects (in primates) are not mediated by opioid receptors. While OHM3295 has low efficacy opioid agonist effects in both primates and rodents (6), another compound in this series, OHM3568 [compound 28 in (3)], has very low opioid efficacy in rodents and very high opioid efficacy (i.e., fentanyl-like effects) in monkeys (9). Thus, there is not necessarily a strong correlation in the behavioral pharmacology of this series of compounds between primate and nonprimate species.

The purpose of the current study was to assess the binding selectivity and behavioral effects of another fentanyl derivative, OHM3507, that had an unusual pharmacological profile in nonprimate species (2,19). In rabbits, OHM3507 appeared to have low-efficacy opioid agonist actions. For example, when administered alone, OHM3507 produced only modest antinociception (39% of the maximum possible effect), and when administered in combination with morphine, OHM3507 partially reversed morphine-induced antinociception and completely reversed morphine-induced respiratory depression (19). Thus, like mirfentanil and OHM3295, OHM3507 had very limited agonist actions and also attenuated the actions of more efficacious μ agonists (e.g., morphine). In light of the novel and interesting effects that were obtained with other compounds in this series, particularly those that appeared to be low efficacy opioid agonists in nonprimate species (e.g., mirfentanil), the present studies were initiated in the hopes of identifying another fentanyl derivative that might have some potential for the treatment of pain. Thus, OHM3507 was evaluated for its binding affinity to each of the three major types of opioid receptors (μ, κ, δ) and also for its behavioral effects in rhesus monkeys using procedures that have been used to assess the effects of other opioids and nonopioids [e.g., (8,15)].

METHOD

Subjects

For binding assays, adult male Hartley guinea pigs (300–350 g, certified viral antibody free; Hilltop Laboratory Animals, Scottdale, PA) were housed individually and maintained on a 12 L:12 D cycle with free access to food (Agway Guinea Pig Maintenance Ration) and water. For behavioral studies, 14 adult rhesus monkeys (*Macaca mulatta*; antinociception, two male, two female; respiration, three male, one female; drug discrimination, three female; and acquisition and performance, three female) were housed individually with free access to water. Subjects used in studies on acquisition and performance were maintained at 85% of their free-feeding weights by banana-flavored food pellets received during experimental sessions, supplemental feeding (Purina Monkey Chow) in the home cage, fresh fruit, and vitamins. All other subjects had free access to food (Purina Monkey Chow) in the home cage and received fresh fruit twice weekly. Monkeys in the drug discrimination study had received 3.2 mg/kg/day (SC) of morphine for several years prior to these experiments and all subjects had received opioids (chronically for monkeys in the naltrexone discrimination study and acutely for other monkeys) in previous studies.

APPARATUS

Drug discrimination. Subjects were seated in Lexan primate chairs that provided restraint at the neck, waist, and feet; during experimental sessions, chairs were located in sound-attenuating, ventilated operant chambers that were equipped with two or three response levers and accompanying red stimulus lights. Chairs were also equipped with a pair of shoes containing brass electrodes, to which brief, 250-ms, electric shocks (3 mA) could be delivered from an AC generator located outside the chamber. Experimental sessions were controlled and data recorded by microprocessors using Med-PC software as well as commercially available interfacing.

Antinociception. For studies of antinociception, monkeys were seated in chairs that provided minimal restraint at the neck, thereby allowing free access to the tails that hung unimpeded from the bottom of the seat. Thermos bottles filled with water (40, 50, or 55 ± 1°C) were used to assess tail-withdrawal latencies. Latency was measured and recorded by an investigator using a push-button switch connected to a microprocessor.

Acquisition and performance. A removable response panel (76 × 71 × 97 cm; Research Equipment Co., Inc., Byran, TX; model LC-1004) was attached to the side of the home cage during experimental sessions. Three translucent response keys (BRS/LVE, press plate model PPC-012) were located on the response panel 50 cm from the cage floor and 11.5 cm apart. Food pellets could be delivered to an aperture (5.5 cm in diameter) located to the right of the right-most key. Experimental sessions were controlled and data recorded by a microprocessor located in an adjacent room.

Ventilation. Subjects were seated in primate chairs that provided restraint at the neck, waist, and arms; during experimental sessions, the chair was located within a sound-attenuating, ventilated chamber. Alternating layers of Lexan plates and latex collars (two of each), as well as a foam cushion, formed the base and minimized gas leakage from the plethysmograph. Air or 5% CO_2 was pumped into the plethysmograph at a rate of 10 l/min and removed with a vacuum pump. Changes in air pressure were measured using a pressure transducer and recorded through a microprocessor. According to calibration with known standards, changes in pressure were transformed to estimates of ventilation: inspirations/min (f); tidal volume in ml/inspiration (V_T); and minute volume in ml/min (V_E).

Procedures

Opioid receptor binding. The procedures for evaluating binding at μ receptors with [^{3}H-[D-Ala^2, N-Me-Phe^4, Gly^5-OH] (DAGO), κ receptors with ^{3}H-ethylketocyclazocine (EKC), and δ receptors with [^{3}H-[D-Pen^2-D-Pen^5] (DPDPE) were similar to those described elsewhere [e.g., (11)]. Briefly, whole brains (including cerebellum) were homogenized in cold 50 mM TRIS HCl pH 7.4 at 50 mg/ml for 40 s using a polytron. The homogenates were preincubated for 40 min at 37°C and centrifuged at 30,000 × g for 20 min. The pellets were resuspended in buffer at 50 mg/ml and incubated for 2 h at 37°C with 1 nM concentration of tritiated ligand ([^{3}H]DAGO, [^{3}H]EKC, or [^{3}H]DPDPE) and 3–7 concentrations of unlabeled ligand (total volume = 500 μl). Homogenates were then diluted in 4.5 ml of buffer, filtered, and washed with 4.5 ml of cold buffer. Membrane-bound radioactivity was measured using a scintillation counter.

Drug discrimination. Subjects received 3.2 mg/kg/day of morphine SC 3 h prior to daily sessions and discriminated between SC injections of saline and 0.01 mg/kg of naltrexone (10). Training sessions consisted of multiple, discrete 15-min cycles with each cycle comprising a 10-min timeout, during which the chamber was dark and responses had no programmed sequence, followed by a 5-min response period, during which stimulus lights were illuminated and a fixed-ratio schedule of stimulus-shock termination was in effect. During the response period, shocks were scheduled to occur every 15 s. Five consecutive responses on the appropriate lever (determined by an injection administered within the first minute of the time out period) terminated the shock-associated stimulus and postponed impending shock for 30 s. Cycles ended after either 5 min or the delivery of four shocks, whichever occurred first. Responses on the injection-inappropriate (incorrect) lever reset the response requirement on the correct lever. During saline training days, saline was administered during the first minute of each cycle; during drug training days, zero to four saline or sham (no injection) cycles preceded a cycle in which naltrexone was administered.

Testing sessions were identical to training sessions, except that: 1) for some tests, saline was substituted for morphine 3 h prior to the session; 2) five consecutive responses on either lever postponed the shock schedule (i.e., no designated correct lever); and 3) increasing doses of drug (morphine, OHM3507, or naltrexone) were administered across cycles so that the cumulative dose increased by 0.25 log unit per cycle. For antagonism studies, monkeys received saline (not morphine) 3 h prior to the session, a single dose of naltrexone during the time out of the first cycle, and increasing doses of an agonist during the timeout of subsequent cycles.

Antinociception. The latency for monkeys to remove their tails from a thermos containing warm (50 or 55°C) water was used as a measure of antinociceptive effect (4,7); monkeys also were tested with a 40°C stimulus; this stimulus typically does not elicit tail withdrawal within 20 s. While subjects were seated in chairs, the bottom 10–12 cm of the shaved tail was placed in the thermos of water, and tail withdrawal latency was measured. If the tail was not removed within 20 s, it was removed manually by the investigator and a latency of 20 s was recorded for that cycle.

Control (predrug) latencies were measured after subjects had been seated in the chairs for a minimum of 10 min. Single-dose time-course studies were determined for OHM3507 using discrete 15-min cycles (10 min time out; 5 min tail-withdrawal latency measurement period) for a total session time of 90 min (six cycles). All other experimental sessions consisted of discrete 30 min cycles (25 min timeout; 5 min latency measurement period) and utilized a cumulative-dosing procedure whereby injections were administered during the first minute of pretreatment periods. Sessions were terminated when the maximum possible effect (i.e., 20 s latency) was observed in all subjects at 50°C (with the exception that for time course studies with single doses of OHM3507, drug was administered up to doses that produced the maximum possible effect with 55°C), or after 90 min, whichever occurred first.

A cumulative dosing procedure was used whereby the dose of agonist increased by 0.25 or 0.5 log unit per cycle. In antagonism studies, a single dose of antagonist was administered 10 (naltrindole) or 15 (naltrexone) min prior to the first injection of agonist. Because the antagonist effects of 0.01 mg/kg of naltrexone (SC) decline markedly after 2.5 h [e.g., (5)], sessions with antagonists were limited to 90 min, or a maximum of five doses of agonist. Control latencies were determined immediately before the administration of antagonist, and again immediately prior to the first agonist injection. Tests were administered no more than twice weekly, with an intervening period of at least 48 h between tests. Other responses (e.g., flushing, pupillary dilation, decreased activity) were also noted and recorded immediately prior to the latency measurement period in the antinociception studies.

Acquisition and performance. A multiple schedule comprising a series of alternating acquisition and performance components (15) was used to evaluate the effects of OHM3507 on a two-member conditional discrimination. Within each component, subjects could respond on the right or left key, with the correct response (key) determined by stimuli that were displayed on a center key (i.e., a combination of four different colors and four different geometric shapes). A correct response resulted in a continuation to the second link of the component, during which a different combination of stimuli were displayed on the center key. A completion of the two-member discrimination resulted in the delivery of a 50 mg food pellet; an incorrect response resulted in a 5-s time out, during which responses had no programmed consequence. During the acquisition component, the correct stimuli varied across days; during the performance component, the stimuli were the same across days. Experimental sessions began with an acquisition component and alternated with a performance component after 20 food presentations or 15 min, whichever occurred first. Consecutive components were separated by a 5 s time out, during which all stimuli were extinguished and responses had no programmed consequence. Sessions terminated after the delivery of 200 reinforcers, or 120 min, whichever occurred first. Sessions were conducted 5 days per week, with drug administered generally on Tuesdays and Fridays (no more than twice per week), and saline (control session) administered on Thursdays. Drug or saline was administered (SC) 10 min prior to the session (i.e., the first acquisition component); for antagonism studies, naltrexone was administered (SC) 30 min prior to the session.

Ventilation. The procedure that was used to study ventilation was similar to procedures described previously (7,14). Subjects were seated in a primate restraining chair that was fitted with the head plethysmograph and located in a sound-attenuating chamber. Experimental sessions consisted of a series of discrete, 30-min cycles, beginning with a saline (control) cycle and followed by two to six cycles during which either drug or saline was administered during the first minute of each cycle. Each cycle comprised a 23-min exposure to air, followed by a 7-min exposure to 5% CO_2. Data were recorded continuously throughout the cycle, and reported as the mean of the last 3 min of exposure either to air or to 5% CO_2. Drug was ad-

ministered no more than twice weekly and with an intervening period of at least 48 h between consecutive drug tests.

A multiple-dosing procedure was used for morphine and for OHM3507, whereby the cumulative dose of drug increased by 0.25 or 0.5 log unit per cycle. Test sessions were terminated when VE was decreased to at least 50% of control in air, or eight cycles (4 h), whichever occurred first. During antagonism studies, a single injection of 0.01 mg/kg of naltrexone was administered one cycle (i.e., 30 min) prior to the cycle during which the first dose of agonist was administered.

Data Analyses

Specific binding was determined to be: (total binding measured) − (binding in the presence of 1 μl of cold ligand). IC_{50}s were estimated by plotting the percentage of specific binding as a function of the −log (inhibitor concentration).

Drug discrimination data are presented as the percentage of responses on the drug-associated lever (% DR [number of responses on the naltrexone-associated lever]/[total number of responses] × 100) and are plotted as a mean value ± 1 SEM as a function of dose. Drugs that produced at least 90% responding on the drug-associated lever were considered to have substituted for the training drug (naltrexone); conversely, drugs that reversed naltrexone-lever responding (i.e., in monkeys that were acutely deprived of morphine) to less than 10% were considered to have substituted for morphine.

Tail-withdrawal latencies are presented as the percentage of the maximum possible effect (% MPE; 20 s) and were calculated as: % MPE = [(experimental latency − baseline latency)/(20 − baseline latency)]. These values were calculated individually for each subject then averaged among all subjects; mean values ± 1 SEM are plotted as a function of dose or time after drug administration.

The effects of drugs on acquisition and performance were determined by calculating the overall rate (i.e., responses/min) and accuracy (i.e., percentage of errors [incorrect responses]/[total number of responses] × 100) for each component. A drug was considered to have an effect when the range of values obtained with a dose of OHM3507 fell outside of the range of values obtained with vehicle. Data are plotted as a mean ± range as a function of dose.

The ventilatory parameters that were monitored and reported were f (frequency), V_T (tidal volume, ml) and V_E (minute volume, ml [f multiplied by V_T]). Measures of ventilation in air and in 5% CO_2 are presented as a percentage of values determined in the absence of drug (% control) during the first cycle of each session and are plotted as a function of dose.

Potency differences among drugs were estimated by comparing ED_{50} (drug discrimination and antinociception) or ED_{80} [acquisition and performance (because response rates were not decreased to less than 50% of control rates under all conditions) and ventilation (because ED_{50} doses were the same or nearly the same as doses that decreased ventilation to the extent that subjects had to be rescued with opioid antagonists)] values that were determined by linear regression, when three or more appropriate data points were available, or otherwise by interpolation. The apparent affinity of antagonists (pA_2 and pK_B) was estimated using the methods of Arunlakshana and Schild (2) as well as Schild analyses with the slope constrained to −1 (18) on the assumption of a simple, competitive interaction at a single receptor type. For some studies, Student–Newman–Keuls t-tests and ANOVA were conducted on ED_{50} values to identify statistically significant differences between conditions.

Drugs

The drugs used in these studies were morphine sulfate, naltrexone hydrochloride, naltrindole hydrochloride, fentanyl citrate (National Institute on Drug Abuse, Rockville, MD), mirfentanil hydrochloride, and OHM3507 hydrochloride [synthesized by L. L. Brockunier according to (3)]. Drugs were dissolved in sterile 0.9% saline, water (OHM3507) or a propylene glycol vehicle (40% propylene glycol, 50% physiological saline, and 10% ethanol; OHM3507 in concentrations greater than 10 mg/ml). OHM3507 was made fresh daily as needed. Drugs were administered IM (acquisition and performance study) or SC (all other studies), typically in a volume of 0.1 ml/kg body weight.

RESULTS

Opioid Receptor Binding

The IC_{50} values obtained for OHM3507 in displacing [^{3}H]DAGO, [^{3}H]DPDPE, and [^{3}H]EKC were 10, 63, and 1764 nM, respectively. Thus, OHM3507 displayed the highest affinity for opioid receptors and the lowest affinity for κ opioid receptors. OHM3507 had a sixfold selectivity for μ receptors over δ receptors and a 28-fold selectivity for δ receptors over κ receptors.

Drug Discrimination

In morphine-treated monkeys, increasing doses of naltrexone occasioned a progressively greater percentage of responding on the naltrexone-associated lever (ED_{50} = 0.009 ± 0.002 mg/kg) with greater than 90% drug-lever responding occurring with doses of naltrexone larger than 0.01 mg/kg (left panel, Fig. 2). When saline was substituted for the daily injection of morphine, monkeys responded at least 90% on the naltrexone lever (point above C, right panel, Fig. 2); under these conditions, morphine dose dependently reversed naltr-

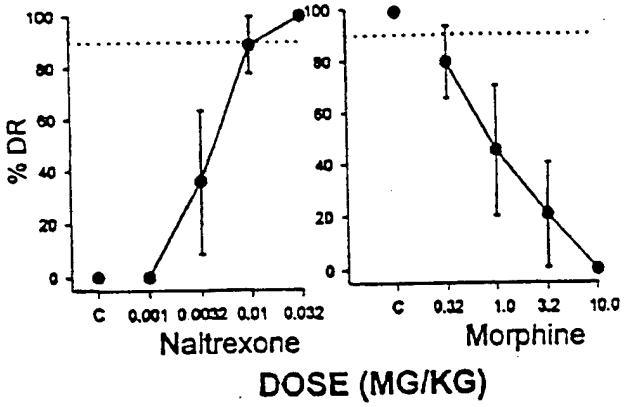

FIG. 2. Discriminative stimulus effects of naltrexone (left panel) and morphine (right panel) in four rhesus monkeys treated daily with 3.2 mg/kg of morphine 3 h prior to sessions in which they discriminated between saline and 0.01 mg/kg of naltrexone. For the naltrexone dose–effect determination (left panel), monkeys received the normal daily dose of morphine 3 h prior to the session; for the morphine dose–effect determination (right panel), saline was substituted for the daily dose of morphine 3 h prior to the session (i.e., monkeys had not received morphine for 28 h). Data are expressed as means ± 1 SEM. Ordinates: percentage of responses emitted on the drug-associated lever (% DR); abscissae: dose in mg/kg of body weight. C = values observed under saline (control) conditions.

exone-lever responding (ED_{50} = 0.94 ± 0.35 mg/kg) with a dose of 10.0 mg/kg of morphine occasioning exclusively saline-lever responding (right panel, Fig. 2). Response rates were not different from control (saline) rates for any dose of naltrexone or morphine (data not shown).

OHM3507 also reversed naltrexone-lever responding in monkeys that were acutely deprived of morphine (circles, Fig. 3); OHM3507 was 6.7-fold more potent than morphine in this regard (OHM3507 ED_{50} = 0.14 ± 0.03). Naltrexone dose dependently antagonized the discriminative stimulus effects of OHM3507, as evidenced by dose-related shifts to the right in the OHM3507 dose–effect curve (Fig. 3). A Schild analysis for naltrexone in combination with OHM3507 yielded a pA_2 of 8.41 ± 0.02 and a slope of −1.20 ± 0.02 (r^2 = 0.99). When the slope of the Schild plot was constrained to −1, the pA_2 for naltrexone was 8.24 ± 0.02.

Antinociception

In the absence of drugs, monkeys never removed their tails within 20 s from a thermos containing 40°C water. In contrast, the average control (baseline) latencies from 50 and 55°C water were 1.62 ± 0.33 s and 1.06 ± 0.19 s, respectively. Single doses of OHM3507 produced time- and dose-related increases in the latency for monkeys to withdraw their tails from 50 and 55°C water (Fig. 4). A dose of 0.01 mg/kg of OHM3507 did not have any consistent effect on tail-withdrawal latency for 90 min after SC administration. A dose of 0.32 mg/kg of OHM3507 maximally increased latencies from 50 and 55°C water; the effects of this larger dose of OHM3507 reached a maximum 15 (50°C) or 30 (55°C) min postinjection and persisted either for the duration of the 90-min test (50°C) or for less than 45 min (55°C). Tail-withdrawal latencies were within the range of control values 24 h after administration of OHM3507 (data not shown).

All four of the agonists studied under a cumulative-dosing procedure increased tail withdrawal latencies in a dose-related manner (data not shown) with the following order of potency: fentanyl (ED_{50} = 0.12 ± 0.01 mg/kg) = OHM3507 (ED_{50} = 0.14 ± 0.05 mg/kg) > morphine (ED_{50} = 1.77 ± 0.64 mg/kg) > mirfentanil (ED_{50} = 7.44 ± 0.59 mg/kg). Naltrexone dose dependently antagonized the effects of OHM3507 on tail-withdrawal latency from 50°C water (left panel, Fig. 5) as evidenced by dose-related shifts to the right in the OHM3507 dose–effect curve. A the Schild analysis for naltrexone in combination with OHM3507 in the antinociception study yielded pA_2 of 7.81 ± 0.03 and a slope of −1.33 ± 0.05 (r^2 = 0.99). When the slope of the Schild plot was constrained to −1, the pA_2 for naltrexone was 8.41 ± 0.02. Naltrindole also antagonized the effects of OHM3507 on tail-withdrawal laten-

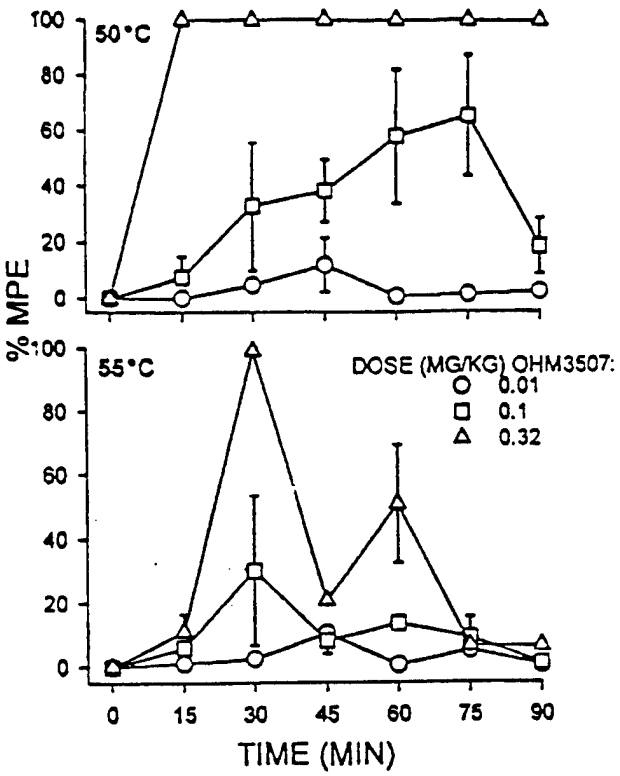

FIG. 4. Time course of effects for single doses of OHM3507 on tail-withdrawal latency from 50° (upper panel) and 55°C (lower panel) water. Ordinates: percent of the maximum possible effect (% MPE) expressed as a mean of four monkeys (except 0.01 mg/kg where n = 3) ± 1 SEM. Abscissae: time, in minutes, after subcutaneous administration of OHM3507.

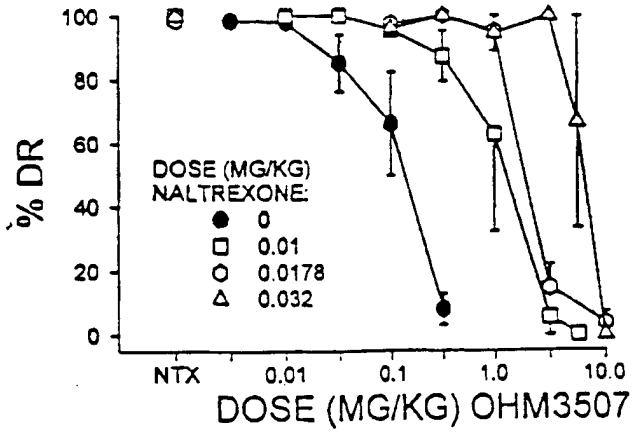

FIG. 3. Upper panel: discriminative effects of OHM3507 in subjects acutely deprived of morphine. OHM3507 was administered alone (circles) and beginning 15 min after an acute injection of naltrexone. See Fig. 2 and the Method section for other details.

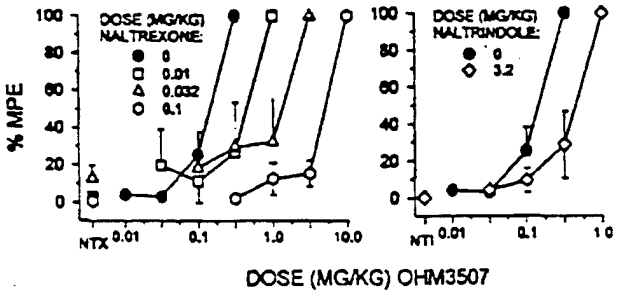

FIG. 5. Effects of cumulative doses of OHM3507 on tail withdrawal from 50°C water. OHM3507 was studied alone (circles) and in the presence of various doses of naltrexone (left panel) or in the presence of 3.2 mg/kg of naltrindole (right panel). See Fig. 4 for other details.

cies with a dose of 3.2 mg/kg of naltrindole shifting the OHM3507 dose–effect curve threefold to the right (right panel, Fig. 5); the pK_B for naltrindole in combination with OHM3507 was 6.5.

Acquisition and performance

Under control (no drug) conditions, incorrect responses in the performance component averaged less than 1 (mean percentage = 0.06 ± 0.03) and in the acquisition component they varied among subjects from 6–23; rates of responding averaged 38.2 ± 0.6 responses per minute in the performance component and 33.3 ± 5.1 response per minute in the acquisition component (Fig. 6). OHM3507 decreased response rates in a dose-related manner at doses that did not significantly affect accuracy in either the acquisition or performance component of the multiple schedule (Fig. 6). In the acquisition component, response rates were decreased to less than four responses per minute at doses of 0.056–0.178 mg/kg. For monkeys CO and BU, OHM3507 was less potent in decreasing responding in the performance component compared to the acquisition component (compare open and closed circles, upper left and upper center panels, Fig. 6); for monkey PA, the potency of OHM3507 in decreasing responding in the two components was the same. With the exception of 0.18 mg/kg in monkey CO (a dose that markedly decreased response rate), the percentage of errors in each of the schedule components was not changed by OHM3507 (open circles, lower panels, Fig. 6).

Naltrexone antagonized the rate-decreasing effects of OHM3507 in both the acquisition and the performance components. Acute administration of 0.032 mg/kg of naltrexone shifted the OHM3507 dose–effect curves for response rate to the right; for all three subjects, the potency of OHM3507 in decreasing responding in the two components was the same when OHM3507 was studied in combination with naltrexone (compare open and closed triangles, upper panels, Fig. 6). The pK_B for naltrexone in combination with OHM3507 was 8.02 for the performance component and 8.23 for the acquisition component. Similar to results obtained with OHM3507 administered alone, the accuracy of responding was not significantly altered by any dose of OHM3507 in combination with 0.032 mg/kg of naltrexone.

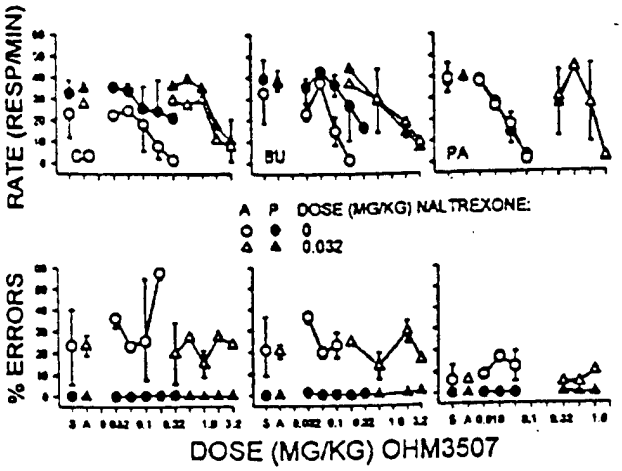

FIG. 6. The effects of OHM3507 on acquisition (A, open symbols) and performance (P, closed symbols). Subjects received either OHM3507 or vehicle 10 min prior to the experimental session. Each set of upper and lower panels shows the effects obtained in individual subjects (CO, BU, and PA). Each data point represents the mean of three determinations in each of the three subjects. OHM3507 was studied alone (circles) and when 0.032 mg/kg of naltrexone had been administered 30 min prior to session (triangles). Ordinates: rate in responses per minute (upper panels) and percentage of incorrect responses (errors) throughout the session. Error bars represent the range of determinations for each condition. Abscissae: dose in mg/kg of body weight. Points above C represent the effects obtained under vehicle control (saline) conditions and points above A represent the effects obtained with naltrexone administered alone.

Ventilation

The control (no drug) values for f, V_T, and V_E in air and in 5% CO_2 are shown in Table 1. On average, exposure to 5% CO_2 increased f, V_T, and V_E to 157, 111, and 171%, respectively, of the values determined in normal air. OHM3507 and morphine (Fig. 7) decreased ventilation in a dose-related manner in all subjects, although there was considerable variability in the potency of both agonists among the four sub-

TABLE 1

VENTILATION IN AIR AND IN 5% CO_2 IN INDIVIDUAL SUBJECTS

	Air			5% CO_2		
Monkey	f (resp/min)	V_T (ml/resp)	V_E (ml/min)	f (resp/min)	V_T (ml/resp)	V_E (ml/min)
MA	37.1 ± 3.7*	35.4 ± 3.9	1169 ± 130	44.9 ± 2.6 (121%)†	38.9 ± 2.9 (110%)	1762 ± 130 (151%)
GO	20.3 ± 1.6	47.4 ± 3.0	942 ± 17	34.6 ± 1.1 (170%)	45.1 ± 3.6 (95%)	1587 ± 118 (168%)
LI	24.2 ± 0.4	45.5 ± 4.1	1108 ± 80	40.0 ± 1.9 (165%)	49.5 ± 1.8 (109%)	1982 ± 130 (179%)
PR	25.9 ± 2.0	29.4 ± 4.5	845 ± 70	44.2 ± 1.9 (171%)	38.4 ± 2.7 (131%)	1577 ± 60 (187%)

*Each entry is the average of nine determinations in each subject.
†Change, in percentage, relative to air.

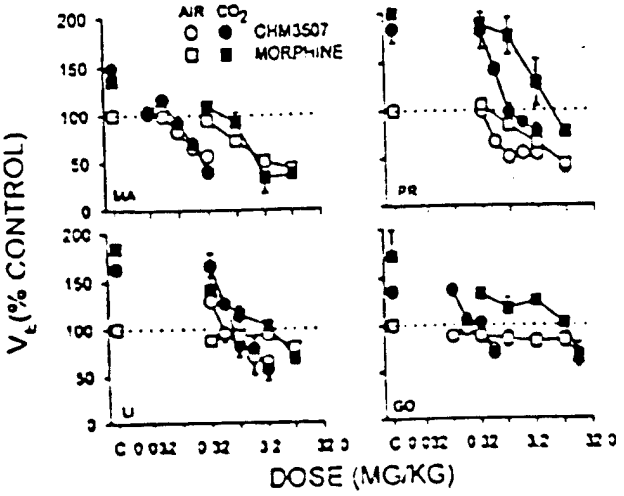

FIG. 7. Dose-effect curves for cumulative doses of morphine (squares) and OHM3507 (circles) on ventilation (V_E) in air (open symbols) and in 5% CO_2 (closed symbols) in four monkeys. Ordinates: averaged V_E expressed as a percentage of V_E under control conditions. Abscissae: dose in mg/kg of body weight. Points above C represent the measures of ventilation obtained under control (no drug) conditions.

jects. OHM3507 decreased ventilation (i.e., V_E) in air and in 5% CO_2 to less than 80% of control at doses between 0.178 and 3.2 mg/kg. In some monkeys (MA, LI, and GO), OHM3507 had a similar potency for decreasing ventilation in air and in 5% CO_2; however, in one monkey (PR), OHM3507 was sixfold more potent in decreasing V_E in air compared to V_E in 5% CO_2 (Fig. 7 and Table 2). The ventilatory-depressant effects of OHM3507 were attenuated by naltrexone, as evidenced by a 2–11-fold increase in the ED_{80} of OHM3507 for decreasing V_E when 0.01 mg/kg of naltrexone had been administered (Table 2). Single-dose affinity estimates (pK_B) for naltrexone in combination with OHM3507 varied from 6.6 to 7.4 (mean = 7.1).

Morphine decreased V_E in air and 5% CO_2 to less than 80% of control at doses between 0.56 and 17.8 mg/kg (squares, Fig. 7). The potency of morphine was similar in decreasing ventilation in air and in 5% CO_2 for three (MA, LI, and GO) of the four subjects, whereas in a fourth subject larger doses of morphine were needed to decrease ventilation in CO_2 compared to ventilation in air.

DISCUSSION

The purpose of the current study was to characterize the binding and behavioral effects of a fentanyl derivative that displayed uncharacteristic effects in preliminary studies conducted in nonprimates species (19). Previous studies with a variety of other compounds in this series, some of which showed novel pharmacological profiles in nonprimate species, have provided strong support for the clinical potential of novel fentanyl derivatives. One of the more compelling results that supports the further consideration of these compounds is that some of them appear to have reduced abuse liability, dependence potential and toxicity, compared to fentanyl, morphine, and related strong μ agonists.

Overall, the behavioral and pharmacological profile of OHM3507 in these studies in nonhuman primates demonstrates that this fentanyl derivative has both high affinity and high efficacy at μ opioid receptors. In binding studies, OHM3507 had high affinity and selectivity for μ opioid receptors, and its overall binding profile resembled the opioid receptor binding profile of mirfentanil under the same experimental conditions (8). The binding affinities of mirfentanil were (IC_{50}s) 27 nM for μ ([³H]DAGO), 262 nM for δ ([³H]DPDPE), and 12000 nM for κ ([³H]EKC) receptors. Thus, the relative affinities of OHM3507 and mirfentanil for δ receptors were only 6.3- and 10-fold less, respectively, than their affinities for μ receptors. Based on results of the binding study, the novel pharmacological actions of OHM3507 might be mediated by δ opioid receptors; however, functional in vivo studies failed to clearly support the involvement of δ receptors (see below). Moreover, the possibility of significant differences between the binding profile of OHM3507 in guinea pig brain and its effects in rhesus monkeys cannot be rejected.

The uncharacteristic behavioral effects of some of the compounds in this series (mirfentanil, OHM3295) results, in part, from these compounds having very low efficacy at μ opioid receptors (e.g., they precipitate withdrawal in morphine-dependent subjects). Moreover, these compounds have limited effects on respiration (in fact, they attenuate the respiratory depressant effects of morphine-like opioids), and antinociceptive effects that are not mediated by opioid receptors (i.e., not blocked by large doses of naltrexone). In contrast, the high-efficacy μ agonist fentanyl has pronounced effects on

TABLE 2

POTENCY OF OHM3507 IN DECREASING VENTILATION IN AIR AND IN 5% CO_2 AND ANTAGONISM OF THE EFFECTS OF OHM3507 BY NALTREXONE

	Air + 0.01 mg/kg			5% CO_2 + 0.01 mg/kg		
Monkey	Control	Naltrexone	Ratio (pK_B)	Control	Naltrexone	Ratio (pK_B)
MA	0.1*	0.3	3.1 (7.3)	0.1	0.4	2.6 (7.4)
GO	0.4	1.0	2.3 (7.4)	0.4	2.3	5.3 (6.9)
LI	1.1	6.5	5.8 (6.9)	1.4	5.0	3.5 (7.2)
PR	0.4	4.4	11.1 (6.6)	2.4	11.0	4.5 (7.0)

*ED_{80} (mg/kg) for OHM3507 in decreasing V_E in individual subjects.

respiration, and its antinociceptive effects are mediated by μ opioid receptors. Although OHM3507 has the same unusual profile of effects in nonprimate species as mirfentanil and OHM3295, this compound in rhesus monkeys appears to be qualitatively identical to fentanyl. In drug discrimination studies, OHM3507 had morphine-like discriminative stimulus effects in reversing naltrexone-lever responding in monkeys acutely deprived of morphine. Like morphine, OHM3507 also had antinociceptive effects in a warm-water tail-withdrawal assay and it decreased ventilatory function in a dose-related manner. Finally, like morphine and other μ agonists, OHM3507 failed to reliably alter accuracy up to doses that substantially decreased the overall response rate in the acquisition or performance component of a complex learning task. Collectively, these in vivo studies fail to show any novel behavioral effects for OHM3507.

One general approach for identifying specific receptor systems that mediate drug effects involves parametric studies with receptor-selective antagonists (8,16). In the current studies, the μ-selective opioid antagonist naltrexone and the δ-selective antagonist naltrindole were used to assess the likelihood that: 1) the effects of OHM3507 are mediated by opioid receptors (i.e., were these effects modified by either antagonist); and 2) the effects of OHM3507 are mediated by a specific type of receptor (i.e., μ or δ). Thus, naltrexone was administered prior to OHM3507 and the dose ratios [i.e., ED_{50} (or ED_{80}) in the presence of an antagonist divided by the ED_{50} in the absence of antagonist] were evaluated using a Schild analysis (1). When the method of drug delivery for the antagonist remains constant across conditions and within a single species, the potency of an antagonist [e.g., apparent affinity (pA_2 or pK_B)] will be the same for attenuating all of the actions of an agonist that are mediated by the same (single) receptor (e.g., μ). Conversely, differences in the affinity of an antagonist among receptor types (e.g., μ and δ) will be indicated by a differential potency (i.e., different pA_2s or pK_Bs) in attenuating the actions of agonists that act at different receptors [e.g., (4)]. Naltrexone dose dependently antagonized the discriminative stimulus, antinociceptive, and response rate decreasing effects of OHM3507 and the affinity estimates that were obtained from Schild analyses were consistent with μ receptor mediation. For example, the apparent affinity estimates for naltrexone in attenuating the discriminative stimulus effects of other established μ agonists [e.g., (9); pA_2 = 8.2–8.6] are not different from the affinity estimates obtained with naltrexone in the current study. However, single-dose affinity estimates (pK_B) obtained with the δ receptor selective antagonist naltrindole failed to clearly support the exclusive role of μ opioid receptors in mediating the antinociceptive effects of OHM3507. The apparent affinity of naltrindole in attenuating the antinociceptive effects of OHM3507 is not different from its affinity in attenuating the rate-decreasing effects of the δ receptor selective agonist BW373U86 (17). Collectively, the qualitative effects of OHM3507 in these well-established behavioral procedures (e.g., reversal of naltrexone-lever responding in monkeys acutely deprived of morphine) as well as the quantitative effects of naltrexone in modifying the actions of OHM3507 (e.g., pA_2 values) provide strong evidence for μ agonist actions of this fentanyl derivative and fail to demonstrate any novel pharmacological features for this compound in rhesus monkeys. Despite the relatively high affinity of OHM3507 for δ opioid receptors in vitro, the behavioral effects of this compound appear to be mediated predominantly, if not exclusively, through μ receptors.

In other species, OHM3507 had novel behavioral actions that were not identical to the effects of fentanyl. There are other examples of a poor predictability between primate and nonprimate species with regard to behavioral effects of opioids. For example, OHM3568 had low efficacy opioid effects as well as nonopioid actions in nonprimate species (3); however, in rhesus monkeys this compound had high efficacy μ agonist actions and was essentially identical to fentanyl (9). Despite the failure of the current studies to confirm a potentially interesting pharmacology for OHM3507 as suggested by previous studies in nonprimate species, additional investigation of other compounds in this series appears warranted in light of the novel, and potentially clinically useful, actions of some compounds in this series (e.g., mirfentanil).

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Effects of Negative Allosteric Modulators of γ-Aminobutyric Acid$_A$ Receptors on Complex Behavioral Processes in Monkeys[1]

J. AUTA, P. J. WINSAUER, W. B. FAUST, P. LAMBERT and J. M. MOERSCHBAECHER

Department of Pharmacology and Experimental Therapeutics, Louisiana State University, Medical Center, New Orleans, Louisiana

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ABSTRACT

A multiple schedule of repeated acquisition and performance of conditional discriminations was used to characterize the effects of two negative allosteric modulators of the γ-aminobutyric acid ($GABA_A$) receptor (ethyl β-carboline-3-carboxylate [β-CCE] and N-methyl-β-carboline-3-carboxamide [FG-7142]), a hallucinogenic β-carboline derivative (harmine), a benzodiazepine receptor antagonist (flumazenil) and a positive allosteric modulator (alprazolam). In the acquisition component, subjects acquired a different discrimination each session. Acquisition of a discrimination was defined by a decrease in errors as the session progressed. In the performance component, the discrimination was the same each session. Responding in both components was maintained by food presentation under a variable-ratio schedule. Incorrect responses in both components produced a 5-sec timeout. Alprazolam (0.1–18 mg/kg), β-CCE (0.01–0.32 mg/kg), FG-7142 (0.1–18 mg/kg) and harmine (0.1–1.8 mg/kg) all dose-dependently decreased response rate in both components. However, accuracy of responding was differentially affected by the drugs. Alprazolam selectively and dose-dependently increased percent errors in acquisition, whereas β-CCE increased acquisition errors only at the highest doses tested in each subject. In contrast, FG-7142 and harmine had no effects on percent errors at doses that virtually eliminated responding. In all cases, performance accuracy was generally not affected. Flumazenil, at doses that had little or no effect (0.1 and 0.32 mg/kg) or occasionally decreased response rates (1 mg/kg) when administered alone, dose-dependently antagonized the rate-decreasing and error-increasing effects of β-CCE, FG-7142 and alprazolam. In contrast, flumazenil failed to antagonize the effects of harmine. Thus, the negative allosteric modulators only moderately disrupted acquisition in comparison with the positive allosteric modulator, but the effects of both types of modulator were antagonized by the benzodiazepine antagonist flumazenil.

The $GABA_A$ receptor is part of a macromolecular complex coupled to a chloride (Cl^-) ionophore. This complex has binding sites for a wide variety of substances from many different chemical classes including the benzodiazepines (Brioni *et al.*, 1989; Fonnum, 1987; Guidotti *et al.*, 1983; Mohler *et al.*, 1987; Saano, 1984; Schwartz, 1988), barbiturates (Fonnum, 1987; Peters *et al.*, 1988; Ticku and Rastogi, 1986) and neurosteroids (Gee *et al.*, 1987; Harrison *et al.*, 1987; Majewska *et al.*, 1988; O'Connor *et al.*, 1988; Perez *et al.*, 1988). Accordingly, $GABA_A$ receptor function can be modulated by different agents within each of these classes and by various endogenous ligands (Sangameswaran and De Blas, 1985). BZDs such as diazepam, triazolam and alprazolam are considered to be positive allosteric modulators by virtue of the fact that they produce an allosterically favorable conformation for GABA binding and thereby enhance Cl^- ion current, whereas β-carboline-3-carboxylates such as β-CCE and FG-7142 are considered negative allosteric modulators or inverse agonists by virtue of the fact that they produce an allosterically unfavorable conformation for GABA binding and thereby inhibit Cl^- ion current (Haefely, 1994; Paredes and Agmo, 1992). Unlike either the positive or negative allosteric modulators, antagonists of $GABA_A$ receptor function (including antagonists that bind to the BZD recognition site such as flumazenil) are generally thought to have little effect on Cl^- channel gating.

Many of the positive allosteric modulators have been shown to be extremely efficacious in the treatment of anxiety (Woods *et al.*, 1992). However, these same high efficacy positive allosteric modulators are also known to produce a large array of unwanted effects such as sedation, potentiation of the effects of ethanol, physical dependence and cognitive deficits (Costa *et al.*, 1994; Woods *et al.*, 1992). Regarding this last effect, BZDs and

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ABBREVIATIONS: GABA, γ-aminobutyric acid; β-CCE, ethyl β-carboline-3-carboxylate; β-CCM, methyl β-carboline-3-carboxylate; FG-7142, N-methyl-β-carboline-3-carboxamide; LSD, lysergic acid diethylamide; BZD, benzodiazepine.

other positive allosteric modulators of $GABA_A$ receptor function are known to impair central nervous system processes involved in the learning (acquisition) and memory (retention) of new information (Cole, 1986; Lister, 1991). For example, the triazolobenzodiazepines such as triazolam and alprazolam have been shown to impair learning and memory in both human and animal subjects (Bickel *et al.*, 1990; Broekkamp *et al.*, 1984; Decker *et al.*, 1990; Lister, 1985; Thiebot, 1985). These same types of deficits have also been shown for other positive allosteric modulators such as thiopental and pentobarbital (Kirk *et al.*, 1990; Moerschbaecher and Thompson, 1980; Osborn *et al.*, 1967), which act at a site independent of the benzodiazepine recognition site. More recent findings have also shown that the partial positive allosteric modulators of $GABA_A$ receptors produce little or no effect on learning and memory when administered alone, but block effects of high efficacy positive allosteric modulators when given in combination (Auta *et al.*, 1995; Thompson *et al.*, 1995). Specifically, Auta *et al.* (1995) found that the combination of either imidazenil or bretazenil with triazolam produced a dose-related attenuation of the disruptive effects of triazolam on two separate behavioral base lines, one involving a learning task and the other involving a memory task.

In contrast to the positive allosteric modulators, relatively little is known about the actions of the negative allosteric modulators or $GABA_A$ receptor antagonists on learning and memory tasks. Several investigators have reported that inverse agonists enhance performance in animals (Chapouthier *et al.*, 1984; Venault *et al.*, 1986) and humans (Duka *et al.*, 1987). Venault *et al.* (1986), for example, reported that pretraining injections of the β-carboline inverse agonist β-CCM increased retention of a habituation test in mice. Raffalli-Sebille and Chapouthier (1991) also reported that pretraining injections of β-CCM enhanced learning of a brightness discrimination task independently of aversive or appetitive motivation. Another β-carboline, ZK 93426, has been shown to block scopolamine-induced amnesia and reverse scopolamine-induced deficits in a signal-detection paradigm (Jensen *et al.*, 1987).

Given the provocative effects reported for the negative allosteric modulators in rodents, the present study was designed to directly compare the effects of a positive allosteric modulator (alprazolam) with two inverse agonists (β-CCE and FG-7142) and a hallucinogenic β-carboline derivative (harmine) on the repeated acquisition and performance of conditional discriminations in monkeys. In addition, the benzodiazepine antagonist flumazenil was administered alone and in combination with both types of allosteric modulator. The same conditional discrimination task as that used by Auta *et al.* (1995) was used in this study to facilitate comparisons between the effects of the positive and negative allosteric modulators. An additional advantage to using this procedure is that the effects of drugs on both learning and performance can be evaluated concurrently. Responding in performance can also serve as a control for nonspecific motivational, sedative, convulsant or muscle relaxant effects of each drug.

Methods

Subjects. Seven adult old-world monkeys served as subjects in these experiments. Subjects I, N and G were female patas monkeys (*Erythrocebus patas*), whereas subjects Co, B and P were female rhesus monkeys (*Macaca mulata*). Subject W was a male cynomlogus monkey (*Macaca fascicularis*). The subjects were housed individually with free access to water, and all subjects were maintained at about 85% of their free-feeding weights on a diet consisting of banana-flavored food pellets (P.J. Noyes Company, Inc., Lancaster, NH), monkey chow, fresh fruits and vitamins. Each subject used in this study had an extensive history of responding under complex behavioral procedures and had been exposed previously to acute drug administration. However, all the subjects were drug free for at least 6 weeks before the start of the present study.

Apparatus. Several removable response panels equipped with response keys and a feeder (BRS/LVE, model TIP-002), the specific details of which have been described previously (Moerschbaecher *et al.*, 1987), were attached to the sides of the individual cages during experimental sessions. Each response panel was connected to a computer and cumulative recorder located in an adjacent room.

Procedure. A multiple schedule of repeated acquisition and performance of conditional discriminations served as the base line for characterizing the effects of all the drugs tested. This procedure, described previously by Moerschbaecher and Thompson (1983), was used to evaluate the effects of the drugs on both the acquisition and performance of a discrimination in a single subject within a single experimental session. In each component of the multiple schedule, subjects were required to respond on a left or right key depending on the stimuli (*i.e.*, different combinations of colors and geometric forms) displayed on the center key. Correct responses resulted in the progression to the next response in the chain in which a different stimulus combination was displayed on the center key. The completion of a chain of these discriminations was reinforced with a 500-mg banana-flavored food pellet. In the acquisition component, the stimuli that set the occasion for left- or right-key responses were changed each session, whereas in the performance component the discriminative stimuli for side-key responses were the same from session to session. Incorrect responses (errors) in both components produced a 5-sec timeout during which responding had no programmed consequences. In summary, in the acquisition component, subjects were required to learn a different discrimination during each daily session, whereas in the performance component the subject performed the same discrimination each session. Each daily session began with an acquisition component, which then alternated with the performance component after 20 food-pellet presentations or 15 min, whichever occurred first. A 5-sec blackout in which all the stimuli were off and responses had no programmed consequences separated consecutive components. Each daily session terminated after 200 reinforcers or 90 min, whichever occurred first.

Drugs. β-CCE and FG-7142 were obtained from Research Biochemical International (Natick, MA). Flumazenil was graciously provided by Hoffmann-La Roche (Nutley, NJ) and harmine (7-methoxy-1-methyl-9H- pyridol[3,4-b]indole) was obtained from Sigma Chemical Co. (St. Louis, MO). Alprazolam was obtained from the Upjohn Co. (Kalamazoo, MI). Harmine was dissolved in sterile water, whereas β-CCE, FG-7142 and flumazenil were dissolved in 5 to 10% dimethyl sulfoxide (depending on the concentration needed) and then diluted with a vehicle containing polyethylene glycol-400 (11%), benzyl alcohol (2%), propylene glycol (50%) and sterile water (37%). For oral administration, alprazolam was suspended in a 2% solution of Suspending Agent K (Bio. Serv. Inc. Frenchtown, NJ) in fruit punch and then mixed (volume, 0.32 ml/kg) with an additional 20 ml of fruit punch, which the subjects readily drank. All other drugs were administered intramuscularly in a volume of 0.05 ml/kg b.wt.; however, at higher doses the injection volume was increased depending on the concentration and solubility characteristics of each drug. The presession administration time for oral administration of alprazolam and flumazenil was 30 min and 15 min, respectively. When β-CCE, FG-7142 and harmine were administered intramuscularly, the presession was 15 min. Intramuscular injections of flumazenil were given 5 min presession.

In all cases, dose-effect curves were determined for alprazolam or

the inverse agonists before any dose combinations with the antagonist were administered. All individual dosages (of agonist or inverse agonist) and subsequent dose combinations with the antagonist were administered in a semirandom or mixed order. Doses of alprazolam or the inverse agonists were frequently given alone, both during and after the antagonist studies, to ensure that the initial dose-effect curves obtained for each drug had not shifted. Because the most pronounced antagonism of the effects of alprazolam and β-CCE occurred with the highest dose of flumazenil tested (*i.e.*, 1 mg/kg), this was the only dose used in the antagonism studies conducted with FG-7142 and harmine in subjects Co, B and P. Drug sessions were generally conducted on Tuesdays and Fridays, with control (vehicle) injections administered on Thursdays. Higher dosages of all the drugs were administered only once a week. Only the highest dose of β-CCE in monkey I (*i.e.*, 0.18 mg/kg) was observed to produce a convulsion. When this occurred the subject was immediately administered 10 mg of lorazepam, which was dissolved in a vehicle of propylene glycol (80%), polyethylene glycol (18%) and benzyl alcohol (2%). These data were excluded from the data analysis for this subject. No convulsions were noted after administration of any dosage of FG-7142.

Data analysis. The data from both components of the multiple schedule were analyzed in terms of the overall response rate (responses per minute, excluding timeouts) and the overall accuracy or percentage errors [(incorrect responses/correct + incorrect responses) × 100]. The data for each subject were analyzed by comparing the range of variability for drug sessions with the control (vehicle) range of variability. Because each subject served as its own control, a drug was considered to have an effect to the extent that the data for a given dosage fell outside of the ranges of variability established during control sessions for that drug. Percent errors were not included in the data analysis when response rate was less than 5 responses/min because of the small number of correct and/or incorrect responses involved. In addition to these measures based on session totals, within-session changes in responding were monitored by the cumulative recorder and computer.

Results

The effects of alprazolam on response rate and percent errors in three subjects are shown in figure 1. Both rate and accuracy in each component for each subject were stable during base-line and control sessions. The rates of responding in both components during control sessions were generally higher for subjects N and I than for subject W. In addition, mean percent errors in acquisition tended to be higher for subjects N and I than for subject W under control conditions. In general, alprazolam produced similar dose-dependent decreases in overall response rate in both components of the multiple schedule in all three subjects. In contrast to the effects on response rate, alprazolam had a more selective effect on accuracy of responding. Alprazolam produced a dose-dependent increase in percent errors in the acquisition component in all three subjects, whereas in the performance component it had little or no effect (compare open and filled circles) except in subject N at the highest dose tested. Note also that there was some differential sensitivity to the disruptive effects on percent errors among the subjects. That is, subject N was less sensitive to the error-increasing effects than subjects I and W. For example, increases in percent errors were evident in subjects I and W at doses as low as 1 mg/kg, whereas a similar magnitude of error-increasing effect was only evident in subject N at a dose of 5.6 mg/kg.

When alprazolam was administered in combination with flumazenil, the dose-effect curves for both response rate and percent errors were shifted to the right (0.5–1 log unit) in all three subjects. This effect is most noticeable at the higher dose of flumazenil in combination with the 5.6 to 18 mg/kg doses of alprazolam. In subject W, for example, 1 mg/kg of flumazenil completely antagonized the effect of 5.6 mg/kg of alprazolam, which was not administered alone because of the substantial effects seen at a lower dose (*e.g.*, 1.8 mg/kg).

The effects on the within-session pattern of responding for subject I after alprazolam (3.2 mg/kg) alone and alprazolam in combination with flumazenil (0.1 and 1 mg/kg) are shown in figure 2. During a control session (top row), the discrimination was acquired during the second acquisition component, and this was characterized by a distinct decrease in the number of errors and an increase in errorless completions of the discrimination. This response pattern in acquisition at the start of the session generally accounted for the fact that the mean percent errors in acquisition for each subject were

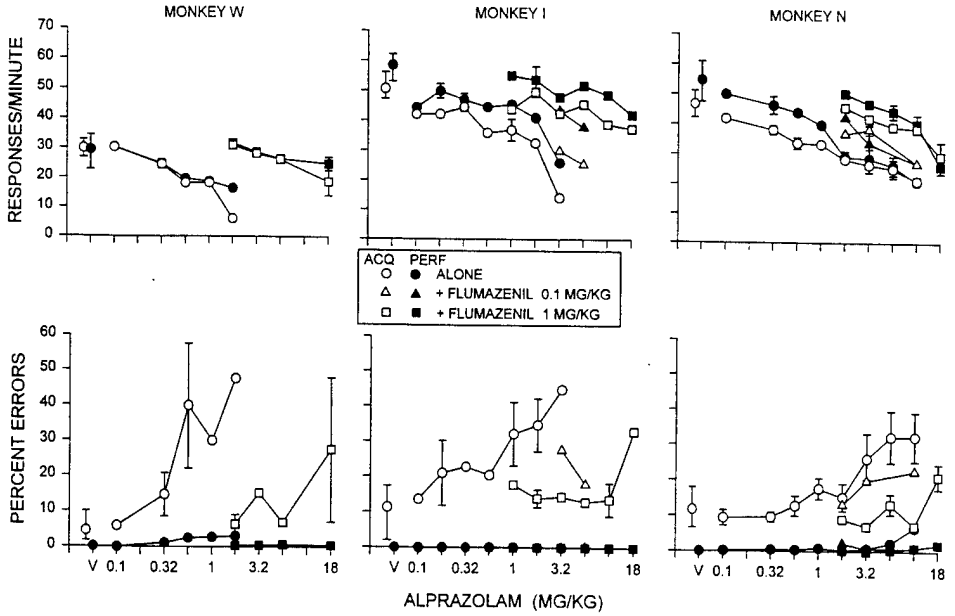

Fig. 1. Effects of oral alprazolam, alone and in combination with orally administered flumazenil (0.1 and 1 mg/kg), on response rate and percent errors in both the acquisition (ACQ) and performance (PERF) components of the multiple schedule in three subjects. Dose-effect data for acquisition are indicated by the open symbols, whereas data for performance are indicated by the filled symbols. Points and vertical lines at V indicate the mean and range of 11 to 14 vehicle (control) sessions. The data points with vertical lines in the dose-effect curves indicate the mean and range of one to two determinations of that dose; data points without vertical lines in the dose-effect curves indicate either a single determination of that dosage or an instance in which the range is encompassed by the point.

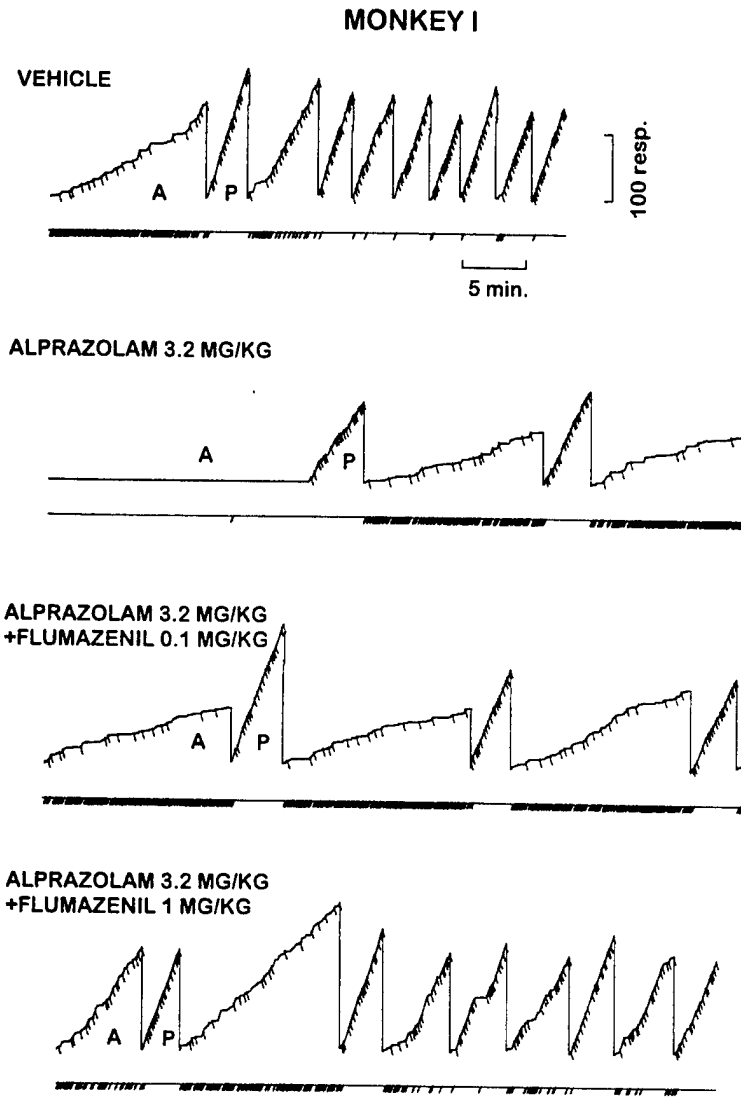

Fig. 2. Cumulative records showing the within-session pattern of responding for monkey I during a representative control (vehicle) session and sessions preceded by the administration of alprazolam (3.2 mg/kg) alone and a combination of this dose of alprazolam with flumazenil (0.1 and 1 mg/kg). The response pen stepped with each correct response and was deflected downward each time food was presented. Errors are indicated by the event pen (below each record), which was held down during each timeout. The event pen was deflected and the response pen reset each time the component of the multiple schedule changed. Each session began with an acquisition component (A) and then alternated with a performance component (P) after 20 reinforcers or 15 min, whichever occurred first. Each session terminated after 200 reinforcers or 90 min, whichever occurred first.

typically larger than mean percent errors in performance under control conditions. When compared with behavior under control conditions, 3.2 mg/kg of alprazolam (second row) selectively decreased response rate and increased errors in acquisition without affecting either measure in performance (compare response pattern between A and P). As shown, this dose of alprazolam completely eliminated responding in the first acquisition component and produced large error-increasing effects in subsequent acquisition components when responding did occur. These error-increasing effects were also evident when 0.1 mg/kg of flumazenil was administered in combination with the same 3.2 mg/kg dose of alprazolam (third row). However, this relatively low dose of flumazenil partially attenuated the rate-decreasing effects as indicated by increased responding in the initial acquisition component and increased responding in acquisition throughout the session. Unlike the lower dose of flumazenil, 1 mg/kg of flumazenil almost completely antagonized the rate-decreasing and error-increasing effects of alprazolam. Note that in the presence of this higher dose of flumazenil, acquisition of the discrimination was evident during the third acquisition component. As in the control record, acquisition was characterized by a decrease in errors as the session progressed and an overall response rate similar to that seen in the performance components. In general, these same effects on the within-session patterns of responding were noted for subjects W and N.

The three panels in figure 3 show the effects on overall response rate and percent errors for three subjects during the acquisition and performance components after injections of β-CCE alone (top panel) and β-CCE in combination with two doses of flumazenil (middle and bottom panels). As in figure 1, the mean overall response rates and percent errors for subject W under control conditions were generally lower than the mean control data for the other two subjects (G and I). Increasing doses of β-CCE administered alone (open and filled circles in the top panel) generally produced dose-related decreases in overall rates of responding in both components in all three subjects. Note that these rate-decreasing effects tended to occur at lower doses in subjects G and I than in subject W. In regard to accuracy in the acquisition component, β-CCE had little or no effect across all doses tested in subject W, but produced marked increases in percent errors in subjects G and I at the higher doses. This was in direct contrast to the effects of β-CCE on the accuracy of responding in performance where the same doses of β-CCE produced little or no increases in percent errors. This was particularly

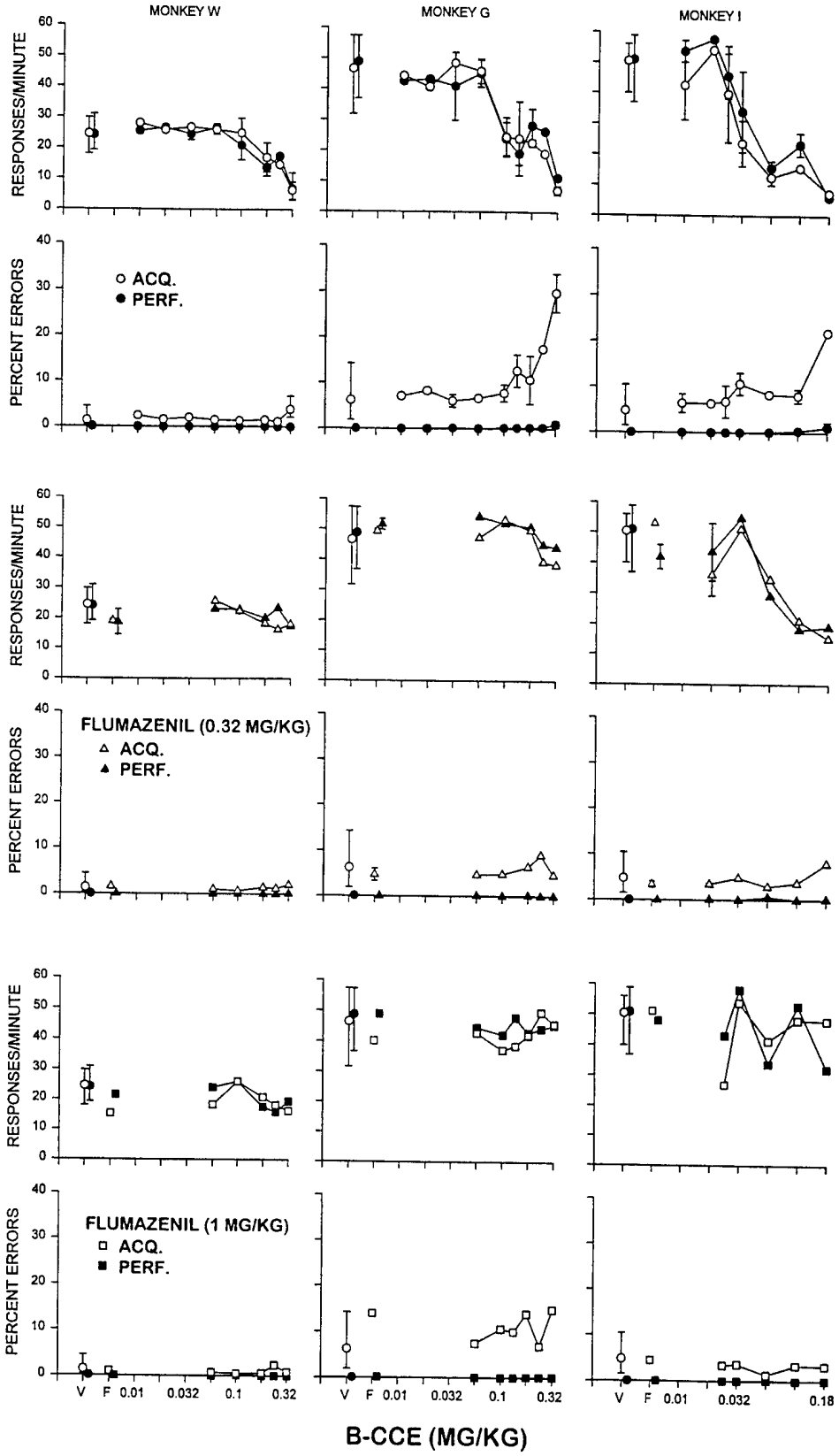

Fig. 3. Effects of β-CCE alone, and β-CCE in combination with flumazenil (0.32 and 1 mg/kg), on the overall response rates and percent errors in the acquisition and performance components of the multiple schedule. The points and vertical lines at V and F indicate the mean and range for 26 to 30 vehicle (control) sessions and at least two determinations for doses of flumazenil alone, respectively. Other details are the same as in figure 1.

evident at the highest doses tested in each subject (*e.g.*, 0.18 and 0.32 mg/kg). Interestingly, these higher doses of β-CCE also reduced rates of responding to less than 10 responses/min in both components. Also, in one subject (monkey I), the 0.18 mg/kg dose and a 0.32 mg/kg dose produced a convulsion. On these occasions, this subject was immediately administered a dose of lorazepam, and the data were excluded from the data analysis.

Unlike β-CCE, flumazenil (0.32 or 1 mg/kg) alone had no effect on overall response rate or percent errors in all three subjects. However, when administered in combination with β-CCE, these same doses of flumazenil dose-dependently antagonized the rate-decreasing and error-increasing effects of β-CCE in each subject. The dose-effect data in the bottom panel of figure 3 clearly show that 1 mg/kg of flumazenil almost completely antagonized the effects of β-CCE on both the accuracy and rate of responding in both components of the multiple schedule.

The within-session pattern of responding for subject I after 0.18 mg/kg of β-CCE alone, and this dose of β-CCE in combination with 1 mg/kg of flumazenil, is shown in figure 4. As indicated by the response pattern in the vehicle record (top row) and the record for 1 mg/kg of flumazenil alone (third row), acquisition of the discrimination occurred a short time after the start of the session, and the pattern of responding in acquisition was similar to that seen in performance for the remainder of the session. Thus, there was generally no difference between vehicle or flumazenil administration in regard to the within-session pattern of responding. In contrast, 0.18 mg/kg of β-CCE alone substantially altered the within-session pattern of responding in both acquisition and performance. These effects of β-CCE were characterized by high initial rates of responding followed by a decrease and then a cessation of responding in both components. This figure also illustrates that the same dose of flumazenil (1 mg/kg) that failed to produce a behavioral effect when given alone, almost completely antagonized the effects of this dose of β-CCE when the two drugs were administered in combination. These same effects on the within-session pattern of responding were obtained in subjects W and G after administration of β-CCE alone and β-CCE in combination with flumazenil.

The effects of FG-7142 on both acquisition and performance are shown for three subjects in figure 5. Although the mean percent errors in subjects Co and B were generally higher than those for subject P, the drug effects obtained were consistent in all subjects. Similar to β-CCE, FG-7142 dose-dependently (but 100-fold less potently) decreased response rate in both components of the multiple schedule, but unlike β-CCE, it did not produce an associated error-increasing effect at doses that substantially decreased overall response rate. These rate-decreasing effects produced by FG-7142 were, in turn, antagonized by a 1 mg/kg dose of flumazenil. Note that this dose of flumazenil shifted the FG-7142 dose-effect curve 1/4 log-unit to the right even though this dose of flumazenil produced small rate-decreasing effects in these subjects when administered alone (see the data at F). This shift in the dose-effect curve could not be determined in subject B due to the difficulty in solubilizing

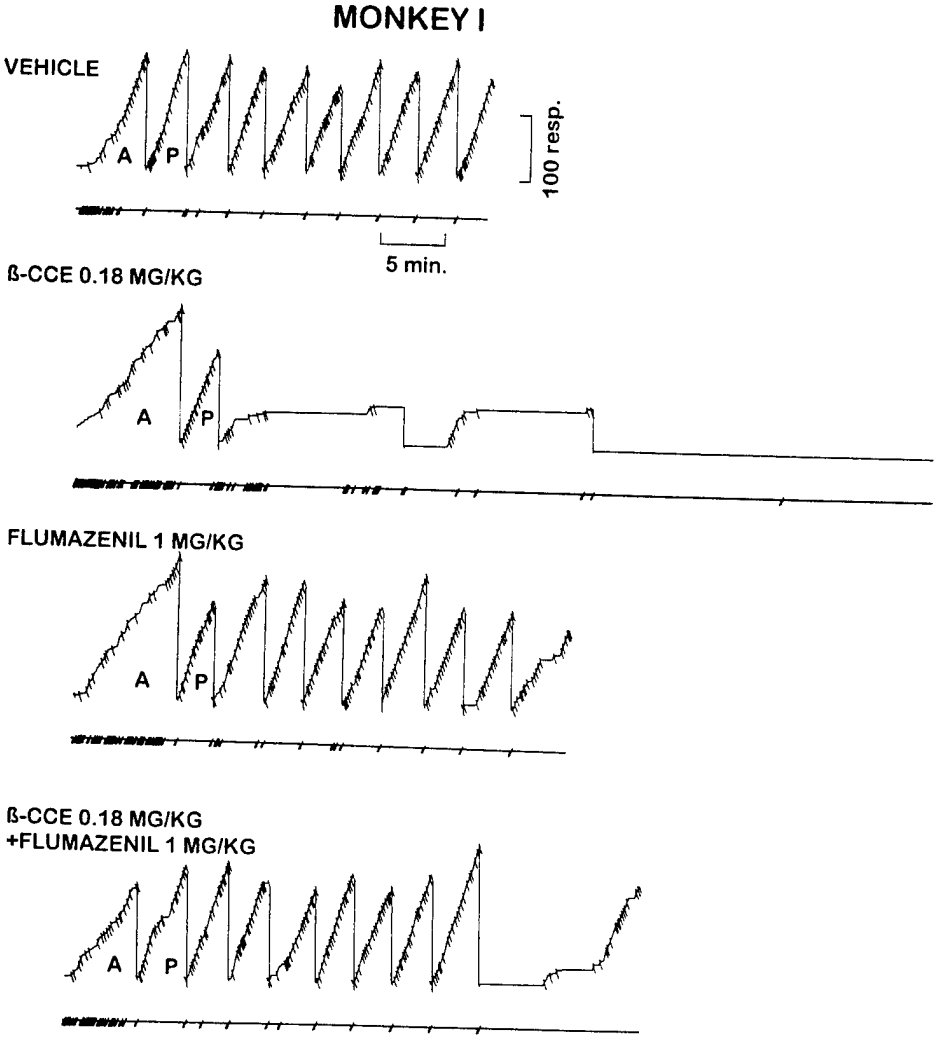

Fig. 4. Cumulative records showing the within-session pattern of responding for monkey I during a representative control (vehicle) session and sessions preceded by the injection of β-CCE (0.18 mg/kg) and flumazenil (1 mg/kg) alone and a combination of these two doses. Other details are the same as in figure 2.

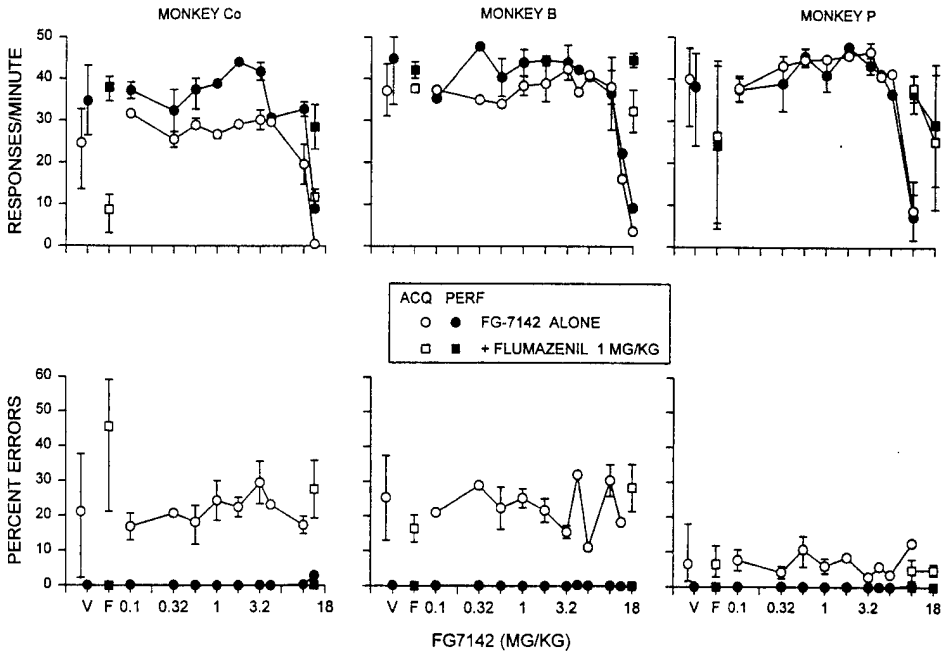

Fig. 5. Effects of FG-7142 on the overall response rate and percent errors in each component of the multiple schedule for three subjects. The points and vertical lines to the left of each dose-effect curve represent the mean and range of 19 to 28 vehicle sessions and 5 to 8 determinations of flumazenil alone.

and administering doses greater than 18 mg/kg. However, 1 mg/kg of flumazenil in this subject completely antagonized the effects of the 18 mg/kg dose of FG-7142.

The effects of harmine on overall response rate and percent errors in each component are shown for three subjects in figure 6. In all three subjects, harmine dose-dependently and uniformly decreased response rates in both components of the multiple schedule. On accuracy of responding, harmine had little or no effect on percent errors in any of the three subjects. The effects on both overall response rate and percent errors were similar to that found with FG-7142 in that harmine failed to increase errors even at higher doses that substantially decreased rates of responding in both components. Unlike the effects of FG-7142, as well as the effects of β-CCE and alprazolam, the rate-decreasing effects of harmine were not antagonized by a 1 mg/kg dose of flumazenil. In subjects B and P, for example, flumazenil failed to antagonize the effects of a 1 mg/kg dose of harmine.

Discussion

The multiple schedule of behavior provided a stable base line with which to examine the effects of each drug on the acquisition (learning) and performance of conditional discriminations, and proved sensitive to the ability of both positive and negative allosteric modulators of $GABA_A$ receptors to differentially affect measures of rate and accuracy within each behavioral component. A consistent finding obtained in the present study concerned the rate-decreasing and error-increasing effects observed when alprazolam was adminis-

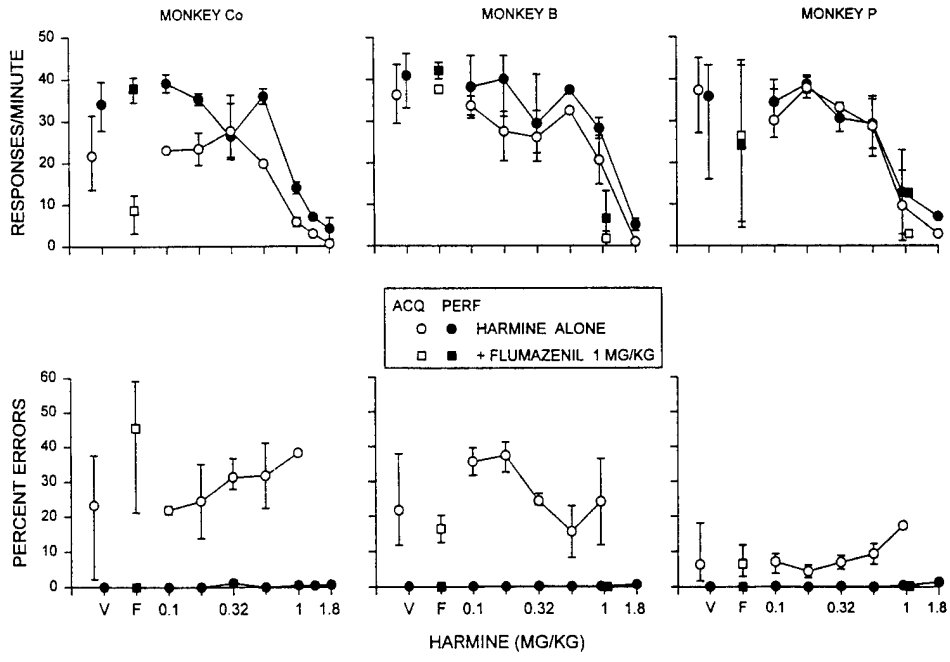

Fig. 6. Effects of harmine on the overall response rate and percent errors in each component of the multiple schedule for three subjects. The points and vertical lines to the left of each dose-effect curve represent the mean and range of 13 to 25 vehicle sessions. The determinations for flumazenil alone are the same as those shown in figure 5. Other details are the same as in figure 1.

tered alone. The dose-dependent disruptive effects of alprazolam on overall response rate and percent errors in acquisition were consistent with previous research concerning the effects of the high-efficacy BZDs on learning and memory procedures in animals and humans (Auta *et al.*, 1995; Thiebot, 1985; Thompson *et al.*, 1995; Woods *et al.*, 1992). Moreover, the selective error-increasing effects produced in acquisition with alprazolam were similar to those found for another high-efficacy positive allosteric modulator of the $GABA_A$ receptor, triazolam, on an identical base line of repeated acquisition and performance of conditional discriminations in monkeys (Auta *et al.*, 1995). In that study, the effects of triazolam were attenuated by either of two partial positive allosteric modulators, imidazenial or bretazenil, when they were administered in combination with triazolam. Thus, data from the present study both replicate and extend these findings by showing that both the rate-decreasing (acquisition and performance) and error-increasing (acquisition) effects of alprazolam could be antagonized dose dependently by flumazenil. Furthermore, these findings are consistent with previous research showing that the amnestic effects of BZD agonists such as diazepam, lorazepam and midazolam are blocked by flumazenil (Ghoneim *et al.*, 1989; McKay *et al.*, 1990; O'Boyle *et al.*, 1983).

One purpose for examining the effects of two negative allosteric modulators of $GABA_A$ receptors under this repeated acquisition of conditional discriminations base line in old world monkeys was to provide a direct comparison with the effects reported for the positive allosteric modulators (Auta *et al.*, 1995). This was particulary important because of the reports which indicated that some inverse agonists enhanced cognition in rodents in several different experimental paradigms used to investigate the effects of drugs on learning and memory. For example, Venault *et al.* (1986) reported that the inverse agonist β-CCM could increase retention in a habituation test in mice. Similarly, other investigators have reported that specific inverse agonists could enhance the learning of a brightness discrimination task in mice (Raffalli-Sebille and Chapouthier, 1991), improve recognition performance of rats after central administration (Mayo *et al.*, 1992) and improve performance of rats in a passive-avoidance task (File and Pellow, 1988; Holmes and Drugan, 1991). Based on these prior results, the suggestion that the inverse agonists might improve learning and memory in monkeys would not have seemed unreasonable. However, the present study found that two inverse agonists (β-CCE and FG-7142) dose-dependently decreased rates of responding while either disrupting or having little effect on accuracy of responding.

The present results in old world monkeys also contrast with a result obtained in humans. In a study conducted by Duka *et al.* (1987), the β-carboline ZK 93 426 was found to improve performance in two cognitive tasks, a logical-reasoning task and a picture-differences task. However, the effects obtained on the logical-reasoning task only indicated a nonsignificant trend toward improvement, and there were some data to indicate that the three groups tested (*i.e.*, placebo and two dose groups) may have had differing performance levels before drug testing. Although the effects reported on the picture-differences task were significant, the data collected for this test were extremely limited in scope. More specifically, no testing was done with this particular task before drug testing to establish comparability among the groups, and the authors only administered this task at one time point after drug administration. Because of the limited nature of the data reported, along with several other important methodological differences (*i.e.*, the drugs themselves, the route of administration and the fact that these authors used solely performance tasks), it is difficult to make any conclusive statements concerning the conditions under which the negative allosteric modulators may or may not facilitate learning and memory. Certainly, under the conditions of our experiment, seeing an improvement in performance would have been difficult because of the already low levels of errors in this highly trained task. However, our purpose for using the multiple schedule of conditional discriminations in old world monkeys was to facilitate direct comparisons between the data obtained here with the negative allosteric modulators and previous data collected with several positive allosteric modulators on the same procedure (*e.g.*, Auta *et al.*, 1995).

Although the effects of β-CCE and FG-7142 on response rates in both components were qualitatively similar to each other and to several other drugs tested, they were quantitatively different from each other. That is, β-CCE was found to be approximately 100-fold more potent on a milligram per kilogram basis than FG-7142. This relatively low potency exhibited by FG-7142 in our study was somewhat unexpected because of the reported similarity in their discriminative stimulus properties (Rowan and Lucki, 1992), anxiogenic properties (Thiebot *et al.*, 1988) and their potency *in vitro* for modulating GABA-induced chloride current (Yakushiji *et al.*, 1989). Even more surprising were the differences found on the accuracy of responding in acquisition after the administration of the higher doses of each drug. More specifically, β-CCE produced increases in percent errors at doses that substantially decreased response rates, whereas FG-7142 produced no increases in percent errors with the same magnitude of rate-decreasing effect. These results obtained largely with subconvulsant doses of each drug suggest that the inverse agonists may be similar to the positive allosteric modulators (and other drugs) in terms of their effects on rates of responding, but dissimilar to the positive allosteric modulators in terms of their ability to disrupt accuracy of responding in a learning task. Although very provocative, a definitive explanation for these differences would be well beyond the scope of this paper and premature given the limited amount of experimental data on the effects of both types of modulator on complex behavioral processes. For example, the different behavioral effects obtained with both types of modulator could occur as a result of the differing distributions of $GABA_A$ receptors across the many regions of the brain that subserve vastly different functions (*e.g.*, motor control *versus* memory). Whereas the differences found between the two negative allosteric modulators could result from differences in the specificity with which each of these inverse agonists binds to the various forms of the receptor, which comprise different subunits (*e.g.*, BZD_1 *versus* BZD_2). In any event, further molecular and behavioral studies with both types of modulator will be required to provide more explicit explanations for the observed behavioral effects.

Flumazenil, up to doses that produced disruptions in response rates (subjects Co and P) and increases in percent errors (subject Co), dose-dependently antagonized the rate-decreasing effects of β-CCE and FG-7142 and the error-increasing effects of β-CCE. The disruptive effects observed

after the 1 mg/kg dose of flumazenil in subjects Co and P were somewhat surprising in that the same effects on rate were not observed in the other subjects, and even in these subjects this dose did not consistently produce disruptive effects in both components. Interestingly, when rate-decreasing effects were obtained (at least in one subject, monkey P), they tended to occur toward the end of the session in a pattern not unlike that observed with doses of β-CCE alone (see cumulative record in fig. 5). There is some existing experimental evidence to suggest that flumazenil may have some properties similar to those of the inverse agonists. Rowan and Lucki (1992), for example, found that the stimulus properties of FG-7142 and β-CCE partially generalized to the stimulus properties of a training dose of flumazenil in a study involving a discriminated taste-aversion procedure. File and Pellow (1985) also demonstrated that flumazenil was capable of producing anxiogenic effects similar to those seen with the inverse agonists in several animal tests of anxiety. Certainly, the data from this study are insufficient to suggest that flumazenil's effects on complex behavioral processes may be similar to those of certain inverse agonists. Only further research with the $GABA_A$ receptor antagonists on learning and memory procedures can answer these questions. What the present data do suggest, however, is that GABAergic mechanisms are involved in the behavioral effects produced by alprazolam, β-CCE and FG-7142 under the present experimental conditions.

The hallucinogenic β-carboline derivative harmine (Naranjo, 1967) also decreased rates of responding in both components in a dose-related manner with little or no effect on accuracy of responding. Furthermore, harmine was found to be 3 times less potent on a milligram per kilogram basis than β-CCE at decreasing response rates. Flumazenil, however, failed to antagonize the effects of harmine, which suggested an action at nonbenzodiazepine receptor sites. Although the behavioral effects and the mechanism(s) of action of harmine have not been well studied, it's effects on rates of responding are qualitatively similar to those reported for the prototype hallucinogen LSD (Berryman *et al.*, 1962; Nielsen and Appel, 1983; West *et al.*, 1982). Because it has generally been accepted that the effects of LSD are mediated *via* the $5\text{-}HT_2$ receptor, one could speculate that harmine may be producing its effects *via* a $5\text{-}HT_2$ receptor-mediated mechanism. However, further studies need to be done to elucidate the mechanism(s) by which harmine produces rate-decreasing effects.

In summary, negative allosteric modulators of $GABA_A$ receptors (β-CCE and FG-7142) produce effects on rates of responding in acquisition and performance that are qualitatively similar to those produced by a positive allosteric modulator (alprazolam) and by a β-carboline derivative not thought to modulate $GABA_A$ receptors (harmine). In contrast to the effects on rate of responding, the accuracy data indicated that the negative allosteric modulators were less disruptive to responding than the positive allosteric modulator, which markedly disrupted the acquisition of conditional discriminations. This was particularly true for the negative modulator FG-7142, which did not decrease accuracy even at doses that substantially decreased overall response rate. Despite the differences in their effects on the accuracy of responding, however, the effects of both types of allosteric modulator were most likely mediated through a benzodiazepine binding site on the $GABA_A$ receptor, because they both produced effects that were dose-dependently attenuated by the benzodiazepine antagonist flumazenil. Moreover, because neither β-CCE nor FG-7142 was observed to increase accuracy or enhance acquisition, these data involving a complex behavioral procedure and old world monkeys failed to support previous data obtained with rodents showing that the negative allosteric modulators are capable of enhancing cognitive processes.

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Send reprint requests to: Joseph M. Moerschbaecher, Ph.D., Department of Pharmacology and Experimental Therapeutics, Louisiana State University Medical Center, 1901 Perdido Street, New Orleans, LA 70112-1393.